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HOW WE RESIST DISEASE

AN INTRODUCTION TO IMMUNITY

BY

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138 ILLUSTRATIONS AND 4 COLOR PLATES



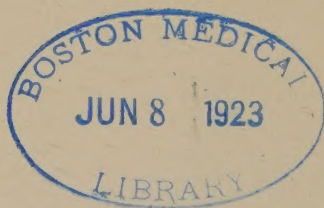
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TO
VERANUS A. MOORE OF CORNELL UNIVERSITY
AND
C.-E.-A. WINSLOW OF YALE UNIVERSITY
TO WHOM I OWE MY
INTRODUCTION TO IMMUNITY

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PREFACE

THIS book, designed as a brief introduction to the exceedingly technical and apparently limitless field of immunity, has been prepared with special reference to nurses and general college students whose programs, ordinarily afford opportunity for but a single brief course in bacteriology, the needs of medical students and those able to devote more time to the subject being already well met by the several excellent and comprehensive textbooks on bacteriology and immunology.

Experience has shown, however, that there is no relation between the time allotted this subject and the range of student interest. This is especially true with regard to nurses, who are interested in so many practical applications of the subject. The aim has been, therefore, to put into fairly simple language the main principles of immunity, covering in a general way the most important preventive and curative practices. To attain this end briefly, without affording opportunity for a large number of attendant misconceptions, is no simple task; realizing this keenly, much attention has been given to the illustrations, not only their number, variety, and range, but their legends as well. It has thus been possible to present a few of the more difficult topics in two—sometimes three ways—the text, the illustration, and the description used with the illustration.

A minimum of space has been given to historical sequences of accumulated facts, except where such treatment emphasized the central or fundamental idea involved, *e.g.*, under vaccines or anaphylaxis. Wherever possible general descriptions of processes, reactions, etc., have been used rather than exact detailed descriptions of the technique involved, as for example in the Wassermann reaction. In all cases, however, the aim has been to give enough detail to enable the student to picture the process or the phenomenon under discussion; this explains such apparent inconsistencies as omitting all mention of the various orders of receptors although complement is discussed in considerable detail.

The terminology has been made as non-technical as possible,

many of the scientific terms being used parenthetically only. Where the avoidance of a technical term would cause a lack of clearness or necessitate the repeated use of a wordy circumlocution such terms have been used, brief characterizations being given in the glossary at the end of the book.

An elementary text is not the place for the weighing of all the various and conflicting theories, such as those advanced with regard to anaphylaxis. While every effort should be made to have the student realize that we are dealing with a growing, developing subject, we must, in such a limited presentation as this, select the theories most generally accepted or the "most workable"—those, which, to the best of our judgment, lead the student most directly and rapidly into this new field, and yet give the beginner a minimum of material which must be unlearned as he advances. This lack of space explains many necessary omissions, such as the interesting theories of Turck regarding the rôle of tissue destruction in disease.

The study suggestions at the end of each chapter are suggestions only, not lesson assignments. In each chapter the memory or review questions might be selected for general class use, the other questions divided among the students who are more interested or have more time at their disposal.

JEAN BROADHURST.

New York, 1923.

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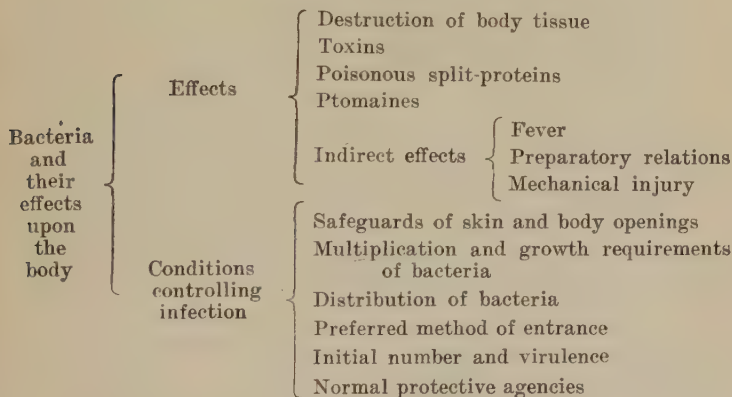
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HOW WE RESIST DISEASE

CHAPTER I

BACTERIA: THEIR ENTRANCE INTO THE BODY AND THEIR EFFECT UPON IT



ALTHOUGH this text presupposes an elementary knowledge of bacteria and their activities, it may be an advantage to introduce this discussion of immunity with a brief summary of the main effects of bacteria upon the body tissues. The efforts of the human body to maintain itself in a state of health, whether by resisting the entrance of such micro-organisms, or by overcoming them after their successful invasion, depend upon and vary with the characteristic activities of the attacking organisms. As indicated in the preceding tabulation bacteria affect the body in many ways; the three most important of these effects are (1) the

destruction of body cells or tissues, (2) the formation of toxins, and (3) the formation of "poisonous split-proteins."

Destruction of Tissue.—Bacteria and other micro-organisms may actually destroy or disintegrate the body cells or tissues. This action is illustrated by some streptococci which dissolve red blood cells (Figs. 1 and 2); by staphylococci, common pus organisms in boils and abscesses, which injure the white corpuscles; and by the "gas bacillus," so prominent in wound infections in the recent war, which destroys muscle tissue very rapidly. Another manifestation of such injurious power is the ability of some intestinal bacteria, such as the dysentery

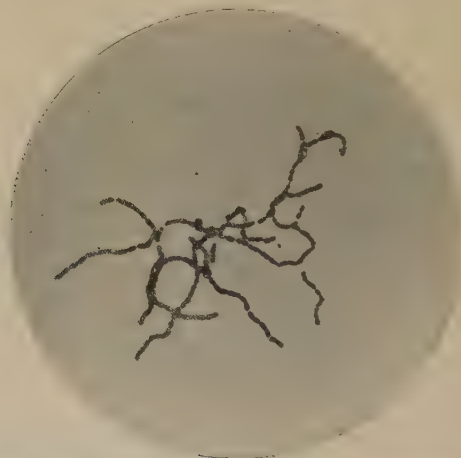


FIG. 1.—*Streptococcus pyogenes*, from chronic suppurating sinus in the upper part of the nose. (x 1200) LEWIS, *Journal of Pathology and Bacteriology*

or cholera organisms, to destroy patches of the mucous membrane lining the intestine, and so allow the absorption of their poisons, which otherwise might pass on out of the body without injury to the individual; this has been demonstrated by experiments in which the animals having uninjured intestinal walls, have been given several hundred times the fatal dose of either live or dead cholera organisms without any ill effects.

Just how these destructive effects are produced is not always demonstrable. Some may be due to specific enzymes. In some cases the effects may be shown to be related to the presence of

toxins and are then attributed more specifically to the toxins, ignoring their probable enzyme character. We are still very far from an adequate comprehension of the activities of bacteria, but it seems legitimate to suppose that enzymes play as varied and important a part in the various powers and manifestations of bacterial cells as in any other plant or animal cells. (See also aggressins, p. 56.)

2. Toxins.—In the ordinary processes of growth of a few kinds of bacteria we are able to demonstrate the formation of

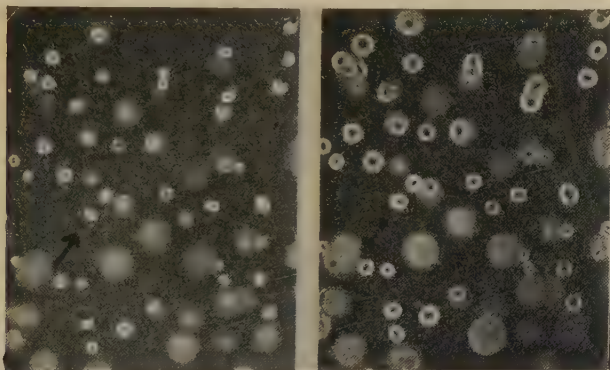


FIG. 2.—Two views of the same region of a blood agar plate showing the power of streptococcus bacteria (*Streptococcus pyogenes*) to destroy the red blood cells. The amount of destruction is indicated by the clear or dissolved area (white in photograph) surrounding each colony. (Horse blood agar plates; Right, longer growing period than Left) BROWN, Monograph, Rockefeller Institute.

special poisonous substances called toxins. These toxins are formed inside the bacterial cells but are soluble and are freely excreted from the cells, so that they pass promptly into the surrounding area (Fig. 10).

The toxins may be locally destructive, destroying the surrounding tissues; or they may be absorbed by the blood circulating through that area and carried through the whole body, irritating or destroying one or more of the more distant body tissues. Less often, as in lockjaw or tetanus, the toxins are absorbed by the nerves rather than by the blood.

Toxins differ from each other not only in the method or path of absorption but also in the tissues they affect. In lockjaw and in botulism the characteristic effects of these organisms are pro-

duced through the nervous tissue. An illustration of more wide-spread effects is provided by diphtheria, in which, besides the local effect—the well-known sore throat—there may be degenerative changes in the liver, supra-renal glands, and the nerve centres combined with extensive changes in the circulatory organs, with the consequent characteristic drop in blood pressure, hemorrhages of the serous membranes inside the body cavity, and degeneration of the heart muscle itself.

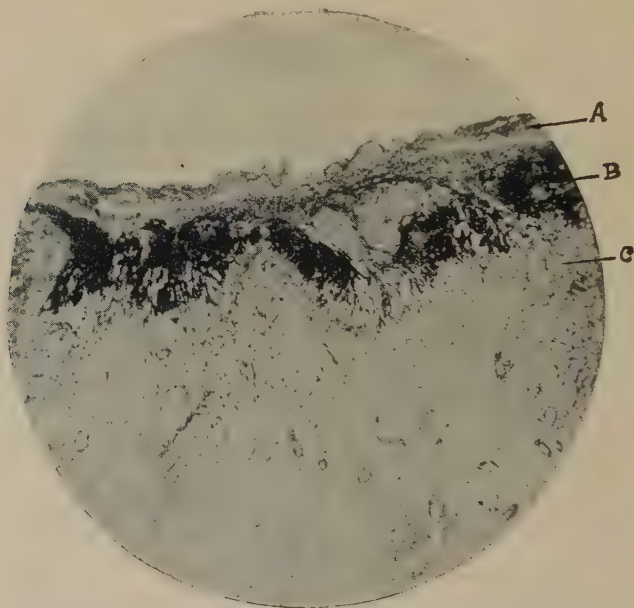


FIG. 3.—Section of tonsil, showing the bacterial layers and necrosis in tonsilitis (Vincent's angina). A, external necrotic layer containing practically no bacteria; B, bacterial layer and necrosis with masses of fusiform bacilli and spiral forms; C, spiral forms in still living tissue. (x90)—TUNNICLIFF, *Journal of Infectious Diseases*.

3. Poisonous Split-Proteins.—The two effects (destruction of tissues and toxins) described above are characteristic of but a minority of the organisms connected with ordinary human diseases. Nearly all pathogenic bacteria owe most of their injurious effects to another cause. In disease these bacteria multiply in uncountable numbers

before our body defenses gain control. Then later as these myriads of invading bacteria are killed and disintegrated, their cell substance is broken down as any protein substance (milk, egg, meat) might be broken or split in ordinary digestion into smaller and still smaller particles. Whether the disintegrating bacteria are in the blood or in tissues richly supplied

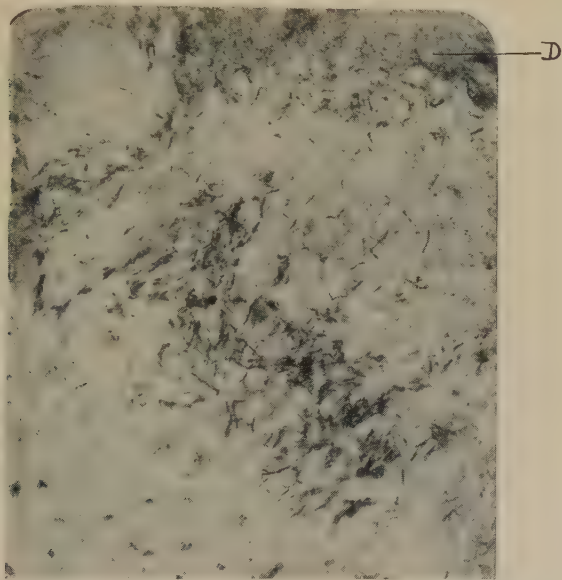


FIG. 4.—Section through the inner edge of B in previous figure (tonsil infection in Vincent's angina) showing more clearly the invasion of living tissue by the spiral organisms. (x575) The organisms are more numerous at D. TUNNICLIFF, *Journal of Infectious Diseases*.

with blood, this disintegration goes on. In this splitting process, certain parts or substances which are poisonous to us are split off or set free. If these poisonous particles (*C* in Fig. 12) are not themselves neutralized or are not further split or broken up into less irritating ones, they accumulate in the body and poison us, being carried everywhere by the circulating blood. Some of these split-proteins irritate one tissue or set of tissues (Fig. 13), some another; therefore, when typhoid bacteria are disinte-

grated we have one set of symptoms, and another set of symptoms characterizes tuberculosis infections (See p. 211).

These substances may correspond to what were formerly called "endotoxins," to distinguish them from the toxins readily excreted from the cell, the extra-cellular toxins already discussed under toxins. While very recent investigations indicate the possible existence of such endotoxins, many of the effects formerly attributed to endotoxins are doubtless due to the disintegration of the bacterial cell proteins—the split-proteins.

Vaughan's most extensive experiments with these split-pro-

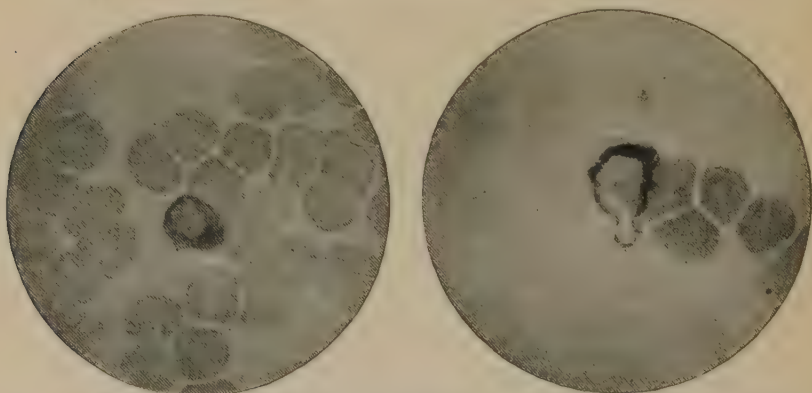


FIG. 9.—Stages in the ingestion and digestion of red blood cells by trypanosomes (*T. Brucei*) isolated from blood of a rat (1250 x). The resulting reduction in the number of red blood cells and in the quantity of hemoglobin is clearly associated with the anemia characteristic of trypanosome infections. SEIDELIN, *Journal of Pathology and Bacteriology*.

teins indicate that they may be obtained from any protein—not only from non-pathogenic bacteria, as well as pathogenic ones, but from ordinary food proteins (eggs, milk) and even from such plant proteins as seeds, by special treatment with acids, alkalies, alcohol, etc. These disintegration poisons or poisonous split-proteins it is clear, then, are not the same as the specific toxins formed by certain bacteria themselves during their ordinary life or metabolic processes (See p. 87).

Though chemists have not yet been able to reduce either the soluble toxins or the poisonous "split-proteins" to exact chemical formulas, there are at least four decided differences between them: (a) True toxins are formed by living cells; poisonous split-

proteins only on the death and disintegration of the bacteria or of similar non-living proteins. (b) True toxins are produced by but few species of bacteria, but the poisonous split-proteins may be obtained on the decomposition of any kind of bacteria or, apparently, any protein substance. (c) True toxins, being soluble and therefore extra-cellular, are found in the filtrate of bacterial cultures, while the poisonous split-proteins are liberated only on the destruction of the cells themselves.

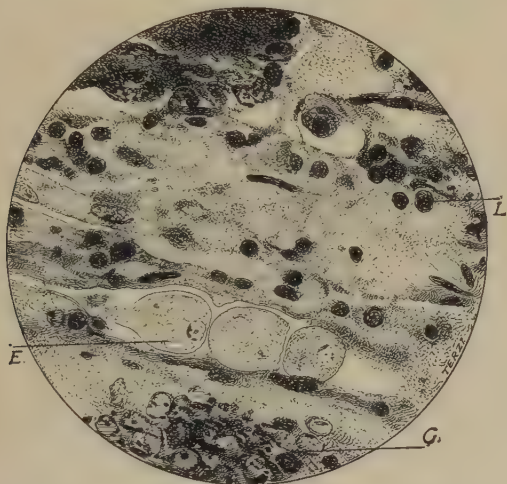


FIG. 5.—Destructive action of *Entameba histolytica*, showing three amebas in cavity caused by their action. (x350) E, ameba; G, disintegrating glandular tissue. We are indebted for the use of this figure to the publishers of "The Essentials of Tropical Medicine," Bale, Sons & Danielson, Ltd., London.

(d) Toxins stimulate the animal body to produce antitoxins (See p. 48); but the poisonous split-proteins, on the contrary, do not excite such antibody reactions. The most that is claimed for the split-proteins¹ is a kind of tolerance, not definite immunity (See p. 207).

4. Ptomaines.—There is still a fourth way in which bacteria are related to disease. Protein foods, in preliminary prepa-

¹No attempt has been made to cover in this brief discussion Vaughan's further differentiation of his split-proteins into "crude soluble poison" and the insoluble non-poisonous "residue," with the differing filterability, of these two parts or fractions.

ration stages or under storage conditions in the shop or home, as well as protein foods actually in the intestine, may be partly digested or broken down by bacteria and ptomaines formed. Most ptomaines are not poisonous; but some of them are very poisonous, and since the power of utilizing protein is a very common one among bacteria, it sometimes happens that the bacteria that happens to get into such protein foods as milk, veal, cheese, fish, and oysters form sufficient ptomaines to poison us. Reliable authorities consider it "doubtful if ptomaines in noticeable quantities are produced within the living infected body."

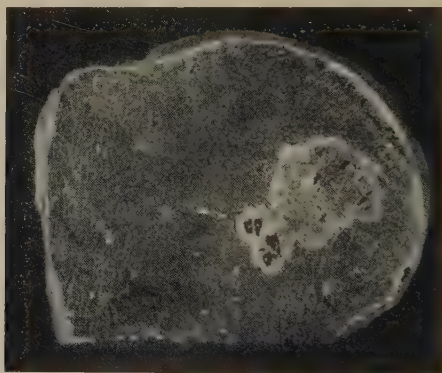


FIG. 6.—A tuberculosis focus or lesion in the head of the femur, illustrating bacterial disintegration of bone tissue. ELY. *International Clinics*, J. B. Lippincott Co.

Ptomaine poisoning is much less frequent than people formerly supposed. Acute intestinal disturbances are more often due to toxic substances produced by the dysentery bacterium or its relatives working in the intestine than to preformed ptomaines in the food eaten. (See auto-intoxication following.)

It was once thought that ptomaine poisoning could be distinguished from bacterial infections in the intestines by the time elapsing between the eating of the suspected food and the development of the attack. Cases developing rapidly (within 24 hours) were classed as due to ingested ptomaines, the ptomaine having been formed before the food was eaten; and the slower

cases were attributed to the taking in of bacteria which continued to develop in the intestine, the symptoms appearing when the bacteria had multiplied sufficiently. Recent experiences indicate, however, that this is not a certain method of differ-

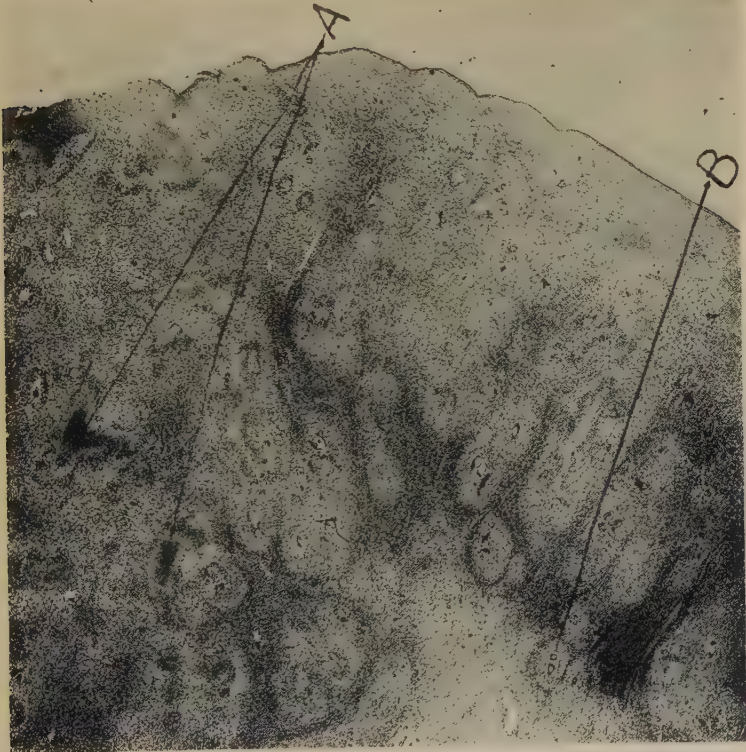


FIG. 7.—Tuberculosis of a synovial membrane magnified about 20 diameters. A indicates an area of necrosis or tissue destruction; at B, note the "giant cells" which are characteristic of certain healing conditions. ELY, *International Clinics*, J. B. Lippincott Co.

entiation, for the rate of multiplication may be surprisingly rapid; the dysentery bacterium, for example, may be responsible for illness developing only 6 to 8 hours after eating food containing these organisms.

Ptomaines are probably more often responsible for less acute and chronic types of intestinal disturbances, auto-intoxication, etc., than for the more acute indispositions such as dysentery and diarrhœa. In such chronic affections, an effort is made to displace the undesirable organisms which continue forming these ptomaines by other organisms; this is often brought about by (1) changes in the diet (feeding milk or lactose, dextrin, etc., Figs. 14, 15, 16) which favor the development of acid-loving organisms such as *Lactobacillus acidophilus* (Fig. 17) normally

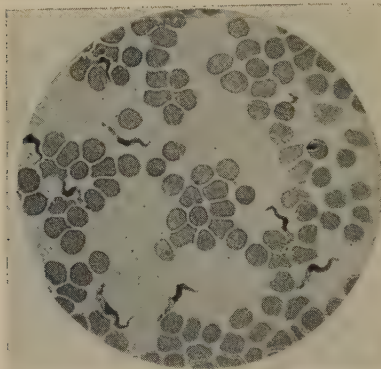


FIG. 8.—Protozoan parasites, *Trypanosoma Brucei*, from blood of a rat. (x1500)

found in the human intestine, or (2) by using sour milk drinks containing acid-forming organisms, such as the lactic acid bacilli or even yeast. Recent experiments indicate that *Lactobacillus acidophilus*, Fig. 17, is much more efficient in this respect than the formerly popular *Lactobacillus bulgaricus* (See Fig. 18), as it readily becomes the most prominent resident of the alimentary canal and *Lactobacillus bulgaricus* does not. Any increase in the acid content of the intestines by either of the above methods, makes the intestines temporarily less alkaline and less favorable for some of the putrefying, ptomaine-forming bacteria.

Ptomaines differ from both of the bacterial poisons, toxins and split-proteins, already discussed, in several ways: (a) The bacterial poisons come from the bacterial cell itself, while the ptomaines are the results of bacterial action on their food material still outside the bacteria, material which has never been part of the bacterial cell, but which is being broken down for use in the cell. (b) The bacterial toxins are all poisons, but most of the ptomaines are not poisonous at all. (c) The chemical structure of the toxins is not known, but the ptomaines are known to be nitrogen compounds of carbon and hydrogen, their poisonous quality varying with their complexity; in the most poisonous ones

we find oxygen as a fourth element. (Putrescin, $C_4H_{12}N_2$, for example has usually but a locally destructive effect, while sepsin, $C_5H_{14}N_2O_2$, is a powerful poison.) (d) No antibodies are stimulated by ptomaines. Therefore, no real immunity can be developed against them, as is the case with toxins. Since the body reactions leading to immunity are our main interest, the ptomaines and their effects will not be further elaborated in this volume.

Other Effects.—To some, this list of bacterial effects would seem incomplete without adding a fifth, namely, fever. Fever,

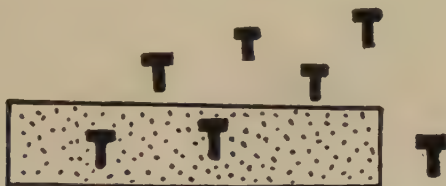


FIG. 10.—A diagrammatic illustration of extracellular toxin in relation to the cell producing it; five toxin particles (*T*) have passed outside the cell membranes, and two are still retained within the cell.

however, must be considered as itself a result of other effects. To prevent the irritation and poisoning already described, the cells of the body react, forming substances to neutralize or destroy the irritating causes. This increased work may be very heavy, and means, of course, increased utilization of food and increased heat production. That in turn demands increased circulation which means still more work with its added heat production. The body temperature changes with the work being done; the rise in temperature is therefore not itself the evil, but an indication of how hard the body is working to overcome the difficulties, to make and distribute the necessary reacting and neutralizing substances. Fever is, therefore, but an index of the patient's condition, and not itself the condition² from which he is suffering.

² Extremely high temperatures, of course, do fix or coagulate cell proteins and it is conceivable that while the fever temperatures fall short of this coagulating point, the cells may be much less plastic or "alive" in the rising temperatures that obtain in high fevers.

Preparatory Relations.—Another important though less direct effect of micro-organisms is shown by a few organisms, which may or may not be harmful themselves, but which act as primary invaders and break down our resistance to other organisms. Recent experiments with rabbits have shown that when influenza exudate from affected rabbits is injected into other animals it may cause lesions in the lung, and may make such animals more susceptible not only to the influenza organisms but to pneumococcus bacteria as well.

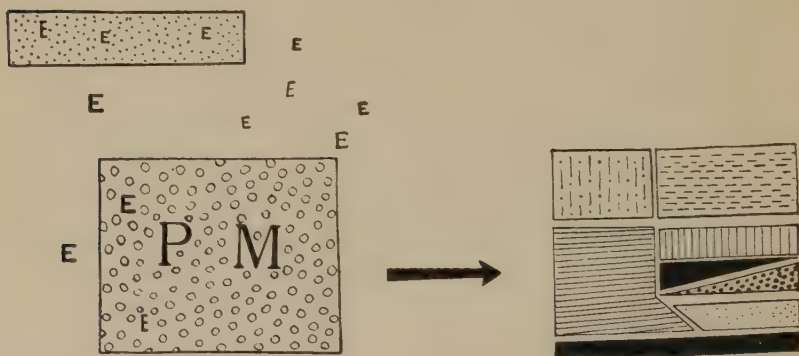


FIG. 11.—Diagrammatic representation of a bacterial cell forming various enzymes, *E*, which are attacking a given protein molecule, *P. M.* in the surrounding food material, causing it to break down into a variety of substances as shown by the diversified drawing on the right; among them may occur one or more ptomaines, which we may assume to be the black products in the right hand figure. This contrasts ptomaines with toxins which, like enzymes, are formed inside the bacterial cells themselves.

To such preparatory action by the influenza organism, or possibly to an as yet undetermined filterable organism³ occurring in influenza infections, is attributed the highly variable character of the recent outbreaks of "influenza" in our country during the war, the number and severity of the cases developing in any locality depending not only upon the resistance to the primary invader but upon the coincidence and virility of other organisms,

³ Since this manuscript was prepared, a filterable organism, named *Bacterium pneumosintes*, has been isolated by Olitsky.

such as the pneumococcus and streptococcus bacteria. This explains such statements as, "Influenza alone is not serious; those

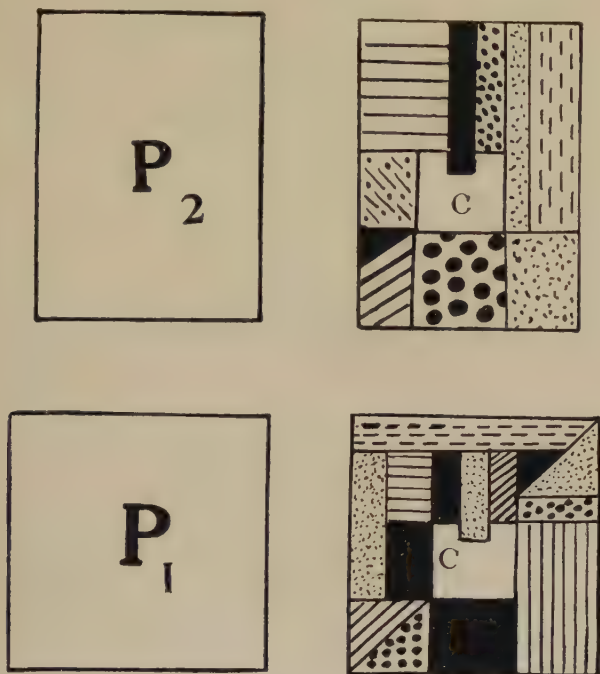


FIG. 12.—Two types of proteins, P_1 and P_2 , showing that each may be broken into various substances, with a similar central core, C , the "poisonous core" of Vaughan.

who die have influenza plus a lung infection—usually a streptococcus or a pneumococcus infection."

Among other bacterial effects which cannot be discussed here is mechanical injury, due to masses of bacteria or bacteria and fibrin which may clog the smaller capillaries and form emboli interfering with the circulation. (See also aggressins, p. 56.)

Paths of Entrance.—The very serious and far reaching effects just described make us ask how it is that our bodies have escaped complete destruction. The answer is that although bac-

teria are very numerous and very widely distributed in nature, relatively few of them really get into the body itself—into the body tissues. The main paths of entrance are the broken skin, the respiratory tract, including the mouth and nose membranes, the conjunctiva and the genital tract, and the alimentary canal. In infections described as originating in the blood stream the primary invasion is really by one of the paths just listed—an unsuspected focus in the tonsils, an unnoticed scratch in the skin, etc.

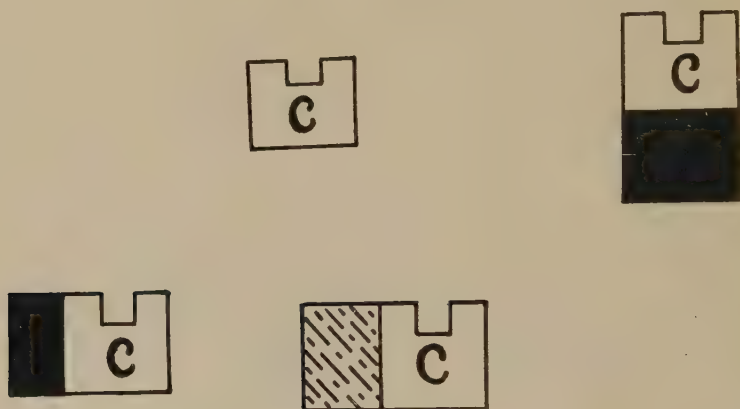


FIG. 13.—The disintegration of a protein may free the "poisonous core" completely, as indicated by the upper left figure. Less complete disintegration might give such results as in the other figures. These differing substances would have varying chemical affinities and so unite or combine with different body substances. This affords an explanation of the specific effects characteristic of the respective bacterial diseases.

The Skin as a Protective Covering.—While such parasites as wriggling hookworms readily penetrate the skin the unbroken skin is not easily invaded by bacteria and protozoa. There is little reason to think that such invasion takes place with ordinary disease organisms unless there are slight lesions or injuries in the skin. Even in animal experiments where bacteria have been proven to enter after being rubbed on the shaven skin, entrance is probably effected through skin lesions that escape the ordinary eye, or through other more or less injured areas, such as might be found in connection with the hair follicles or sebaceous glands.

Without the existence of such lesions bacteria remain on the surface of the skin, entering only when a cut or injury affords them entrance. As some one has well phrased it, "The skin is inhabited but not infected."

Safeguards at Entrances into the Body.—Special safeguards are provided at the places where the ordinary outer skin is replaced by more delicate and thinner coverings, such as the eye and mouth. In each of these areas there are usually present one

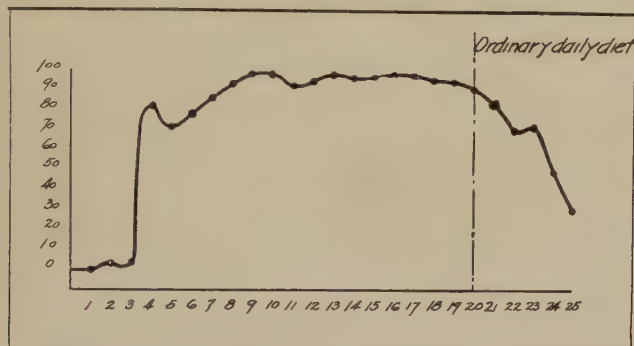


FIG. 14.—This curve indicates the percentage of acidophilus organisms appearing in the fecal specimens of human subjects on ordinary diet plus a milk culture of acidophilus, 1000 c. c. Notice after 20 days, the drop when ordinary diet was resumed. RETTGER AND CHEPLIN, *Intestinal Flora*, Yale University Press.

or more characteristic types of bacteria, apparently (or at least usually) harmless ones, which very rarely penetrate below the surface.

The mouth has many characteristic organisms, streptococci, and other cocci being the types most commonly present, probably. A diphtheria-like bacillus, *Corynebacterium xerosis* (*Bacillus xerosis*, Fig. 20), is characteristically present in the conjunctiva of normal eyes; and similarly other harmless bacilli may be expected in the genital area. Such organisms may possibly help by interfering with the multiplication of ordinary pathogenic bacteria; for instance, the presence of staphylococci in the mouth has been thought somewhat incompatible with that of diphtheria, and some doctors have used staphylococcus in efforts to replace the diphtheria organisms in diphtheria carriers. These regionally characteristic organisms may, however, be disregarded at least

as far as definite immunity is concerned, and our attention may be turned to the special conditions that safeguard such delicate membranes from definitely harmful organisms.

The Eye Safeguards.—The eye membranes are very susceptible to infection at and for a few days following birth. Later, they are much less easily invaded by ordinary bacteria; one has only to recall their exposure to dust, and the very common habit of rubbing the eyes with unwashed or soiled fingers, to be surprised at the comparative infrequency of eye infection. This

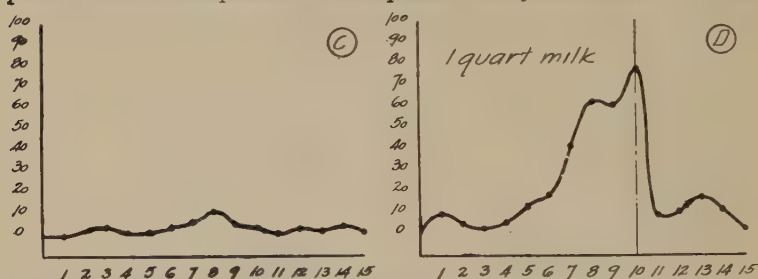


FIG. 15.—Curve indicating the percentage of acidophilus organisms appearing in human subjects on ordinary diet, (C), and with ordinary or untreated milk added to the diet, (D). Note the drop when milk is no longer added. RETTGER AND CHEPLIN, Yale University Press.

freedom is mainly due to constant washing of the eye by the tear fluids, which mechanically remove organisms from its surface. This fluid has besides a slightly germicidal power.

Recently, the eye has been described as a possible route for infections of the nasal tract, including the naso-pharynx. This theory is of special interest in connection with the spread of influenza.

Safeguards in Connection with the Respiratory Membranes.—Besides the varied and more or less resident flora in the healthy mouth, the mouth, nasal and lung membranes are subject daily to the additions of large numbers of bacteria from infected teeth and tonsils, only too often more numerous than it is pleasant to contemplate. (Most of these pass with the saliva into the stomach, but small quantities must be constantly distributed over the mouth and throat membranes.) Both the saliva and the nasal secretions are weakly germicidal. These liquids constantly wash the adjacent membranes, mechanically removing accumu-

lating bacteria. The fetid condition obtaining in the mouth, when the salivary flow is diminished, may be due as much to the lessened mechanical removal of bacteria, as to the inhibitory effect of the saliva. It has been estimated, however, that over 300,000 bacteria are daily taken into the lungs under ordinary conditions. Caught on the moist lung surface, all bacteria must remain there; yet very few lung infections develop compared with the total of daily opportunities.

This is no doubt in great part due to the fact that some of the alveolar cells resemble the white corpuscles in their power of surrounding and destroying bacteria. (See also p. 141.) Then, too, when bacteria do penetrate this thin alveolar membrane, they find themselves immediately in the blood stream, in contact with white corpuscles and special blood substances (antibodies) both of which, as will be shown later, have the power to destroy bacteria.

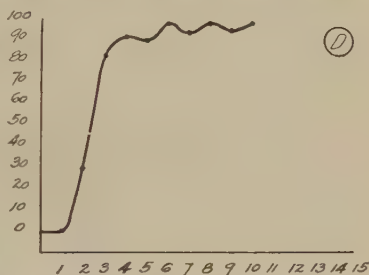


FIG. 16.—Typical curve indicating the percentage of acidophilus organisms in the fecal specimens of a human subject fed on ordinary daily diet plus 300 grams of lactose. RETTGER AND CHEPLIN, *Intestinal Flora*, Yale University Press.

Resistance of the Alimentary Canal.—The alimentary canal furnishes a most striking example of the protective function and resistance of delicate epithelial cells, for surgical work on the mouth and anus heals satisfactorily without the use of disinfectants, in spite of the fact that these regions are constantly exposed to infections.

It is stated that bacteria compose fully one-third of the fecal matter excreted daily. The inner surface of the intestines must be thickly coated with bacteria, even when the intestines are empty of food. Yet in health, the thin lining layers successfully withstand the invasion from the intestine into the deeper tissues of the body cavity. True, these absorbing areas of the intestine are richly supplied with blood containing antibodies, but their success as protective agents is none the less wonderful. One has only to compare the flavor of a freshly killed chicken with a cold-storage one to realize how effective these lining layers are

during life, for the problem of "cold storage chickens" is how to prevent intestinal bacteria from passing through into the flesh, and causing the changed flavor we all know too well.

Further illustration of such difference in resistance between living and dead cells is found in the digestion of a given tissue by the normal digestive juices of that region after death; *e.g.*, in life the mucous membrane of the stomach resists the action of pepsin in the gastric juice, but after death this stomach membrane may be digested by pepsin.

Protective Action of Digestive Juices.—In addition pro-

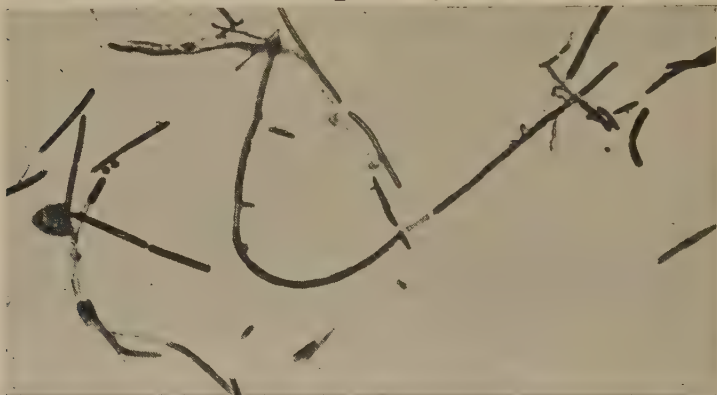


FIG. 17.—*Lactobacillus acidophilus*, 48 hours, glucose broth culture, showing great range in size and chain formation (x1000), RETTGER AND CHEPLIN, Intestinal Flora, Yale University Press.

TECTIVE effects are claimed through the action of such digestive juices as the hydrochloric acid of the stomach, and the intestinal juices, including the bile. The protective action of these juices has probably been overrated, and many reliable authorities ascribe little or no germicidal value to the intestinal secretions. With regard to the acid content of the stomach, the short period of contact, since food may begin to leave the stomach in twenty minutes and water almost immediately, makes it improbable that objectionable bacteria are necessarily subjected for a sufficient period to the hydrochloric acid. Besides, the acid occurs in very dilute amounts—only two-tenths of one per cent.—and experimental work has shown that such organisms as streptococci can withstand at least six times that concentration (one and two-tenths

per cent.) for over an hour. Experiments have established that the organisms of tuberculosis, typhoid and dysentery readily pass beyond the stomach when swallowed with food. Bacterial inhibition, rather than destruction, is all that it is safe to claim for hydrochloric acid in the stomach.

There are, however, other protective influences to be attributed to the stomach region, for although some poisons, such as those found in decayed meat, are not destroyed in the stomach, the poisons of organisms such as tetanus and diphtheria are

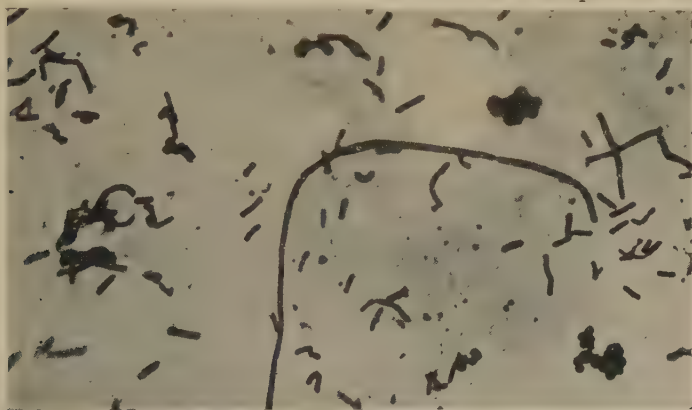


FIG. 18.—*Lactobacillus bulgaricus*, 48 hour whey broth culture, showing close similarity to *Lactobacillus acidophilus*. RETTGER AND CHEPLIN, Intestinal Flora, Yale University Press.

changed by gastric juice. This may have a bearing on the relative freedom of the stomach from bacterial inflammations.

Although the bile is considered somewhat germicidal, disease organisms (typhoid, in typhoid carriers) have been found growing in the bile ducts and gall bladder; the antagonistic action of the bile, therefore, can not be so great or so universal as once thought. Nevertheless, the gastric and intestinal juices including the bile, may have an inhibiting action on the growth of many organisms, and the alternation of an acid and an alkaline medium (stomach to intestine) is thought to be somewhat helpful in reducing the kind and number of bacteria found in the intestine.

The preceding paragraphs have indicated the difficulties met by organisms in getting past the external barriers into the tissues. But even when they do succeed in effecting an entrance, infection

does not always follow. There are two possible reasons for this: (1) The bacteria may not find conditions there that favor their growth or (2) they may be met by definite antagonistic substances that destroy them.

Multiplication Necessary to Establish Infection.—

Ordinarily very few pathogenic bacteria enter the body at any one time—in a glass of water, in human-polluted air, on handled food, or through a cut. One or even a few bacteria can not cause infection usually. (See p. 43.) They may be disposed of at once by the protective forces (white corpuscles and antibodies) to be discussed later.

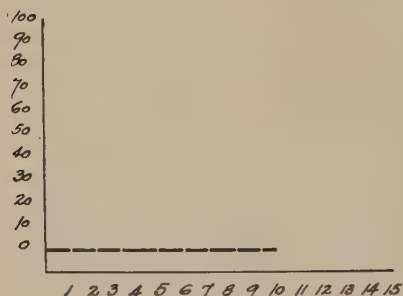


FIG. 19.—Compare this typical result when broth cultures of the *bulgaricus bacillus* are fed with ordinary diet and note that, unlike acidophilus, the *bulgaricus* organism produces no apparent change in the intestinal flora. RETTGER AND CHEPLIN, Intestinal Flora, Yale University Press.

Even if the few organisms entering escape destruction, multiplication is also necessary for infection to develop. This means that the environment must be favorable for the invading organism—that it must find itself supplied with the right food materials, suitable amounts of oxygen, favorable temperature, etc.

While the human body has been aptly called “the greatest living culture medium and incubator,” most of

the hundreds of types of bacteria known to us do not find it favorable for their growth and multiplication.

Microscopic observation has shown that under favorable conditions, some organisms may grow to full size and form two new ones in twenty minutes. At this rate one organism could produce over 2,000,000 bacteria in eight hours. It has been estimated that if streptococci multiplied in our bodies without hindrance, one initial organism could cause death in 18 hours; a single tuberculosis cell, in three weeks. Fortunately, disease organisms rarely advance at such rates, and as mentioned earlier, many bacteria fail to multiply in the body at all. Out of several thousands of bacteria and protozoa known to science, apparently less than a hundred kinds can grow in the human body with sufficient ease to be considered causes of disease.

How sensitive bacteria are to environmental conditions such as temperature, oxygen and food supply is shown in the following illustrations:

1. *Temperature*.—The various types of tuberculosis organisms (Fig. 21) have very different temperature requirements. On culture media the human type grows best at 37° C. and growth ceases at 40° to 41° C.; the avian type requires a higher temperature than the human, multiplying rapidly at 45° or even higher. As might be expected, therefore, human tuberculosis does not develop in fowls, which have an average temperature much higher than in man, 40° or even higher; and avian or fowl tuberculosis with its higher temperature requirement does not develop in man.

2. *Oxygen*.—*Tetanus* develops best in regions poorly supplied with oxygen, and so ordinarily multiplies in areas poorly supplied with the oxygen-carrying blood, such as the subcutaneous tissue.

3. *Tissue Conditions of Host Cells*.—Experiments have shown that bruised tissue is especially susceptible to bacterial invasion and sometimes, apparently is absolutely necessary for the development of infection. Tetanus bacteria

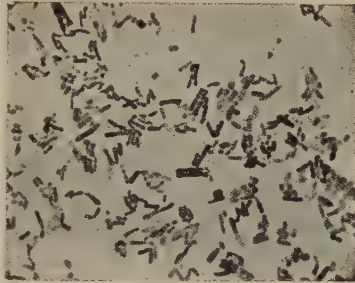


FIG. 20.—A diphtheria-like bacterium *Corynebacterium xerosis* (*Bacillus xerosis*) frequently found in normal eyes. (x) HISS AND ZINSSER, *Textbook of Bacteriology*, Appleton.

spores may be injected into the blood vessels of a guinea pig without causing disease, and spores can be demonstrated in such tissues as the liver and spleen, thirty and even fifty days afterward. But if the tissues are bruised, tetanus develops promptly, beginning in the bruised tissues. (See also resistance, p. 53.) Any necrotic area, or even a blood clot or bruise, may provide the necessary nutritive conditions for the development of tetanus organisms. This may be not only a question of food material, but rather of breaking down the natural cell resistance, for the normal cell resistance is typically high. As Zinsser expresses it, "Invasion of one cell by another is not Nature's plan." The resistance to bacterial invasion shown by cells such as those

lining the intestine is illustrated by the resistance to decay in unprotected living eggs, such as those of the frog. This normal resistance of living tissues is emphasized by the rapid decay which takes place when such eggs die.

Saprophytic and Parasitic Organisms.—The various types of organisms (*e.g.*, staphylococcus) commonly found on the skin are of little importance except as their persistence may afford opportunity for their later entrance into the body, *e.g.*, through a break or cut in the skin.

It is customary to speak of bacteria as either parasitic (de-

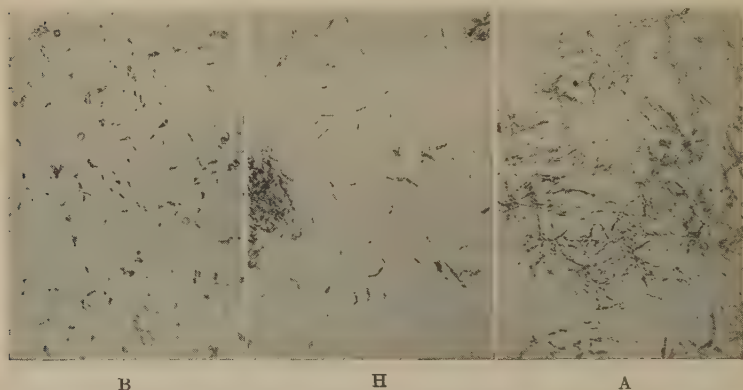


FIG. 21.—Left to right, bovine, (B) human, (H) and avian, (A) tuberculosis organisms, egg-agar cultures. (x1500) SANDS.

iving their food materials from living animals or plants) or as saprophytic (deriving their sustenance from non-living things such as dead animals or plants, milk, bread, leather, etc.). Many of the kinds of bacteria found in the human intestines are not really living on the body tissue, but on the foods they contain, just as they would live and multiply on such foods before they were eaten if the temperature were favorable. It is not always easy, therefore, to know when the terms parasite and saprophyte should be used in speaking of organisms obtained from the body.

Distribution of Bacteria in the Body.—The effects brought about by bacteria are more clearly understood if we know something of their distribution in the body. Though their location may be limited to a very small area, as in small skin boils or abscesses or the throat patches in diphtheria, the infected area

is often a much larger or a more general one, such as the lungs, or the peritoneum.

Even in disease they may be located only in what are really surface tissues. In suppurating tonsils, stomach ulcers and even in the typhoid invasion of glandular areas of the intestine (Fig. 22) the organisms have not penetrated far into the body tissues. Organisms do penetrate into the body, however, sometimes making their way inward by continued disintegration from a surface lesion.

Organisms from a localized site or focus may find their way into the blood stream in considerable numbers. This is true in

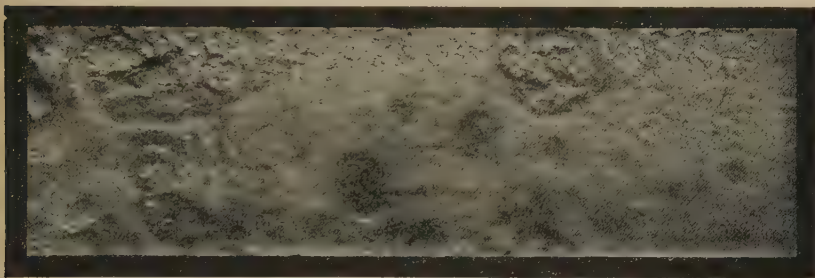


FIG. 22.—Peyer's patches, from intestine of typhoid patient. The swollen patches and nodules are necrotic (disintegrated) tissue except at their edges, the central portions being "ragged sloughs," DELAFIELD AND PRUDDEN, Text book of Pathology, Wood.

diphtheria, where a very definite localized area is ascribed to the causal organisms; similarly, typhoid bacteria are commonly found in the blood in the early stages of typhoid fever. This condition is termed a bacteremia, and the presence of the organisms in the blood is temporary only, for they are soon destroyed by the protective agencies also present in the blood. Pyemia is used to indicate the condition when the organisms thus discharged into the blood and distributed by it cause several different foci. Sometimes the organisms multiply in the blood stream itself (septicemia, Figs. 23, 24). A heavily discharging focus may, of course, approximate this last condition, septicemia, and be difficult to distinguish from it, both in the blood picture presented and the condition of the patient.

In health, occasional bacteria are doubtless constantly making their way into the blood stream, not only from an adjacent focus,

such as an infected tonsil, but through apparently healthy membranes or tissues such as the intestinal wall; but finding no favorable site do not produce infection (See p. 43) before they are destroyed by the white corpuscles or specific antibodies. The white corpuscles are themselves sometimes responsible for this transfer of bacteria to new areas, for they sometimes carry bacteria with them as they occasionally make their way through the capillary walls into the surrounding tissues. Usually, however, they destroy all ingested bacteria and no infection occurs.

The extent of the focus does not necessarily correspond to the effects caused. A very small focus, such as an infected tooth

(Figs. 25, 26) may have serious systemic effects. In tetanus, where we often find but a small focus at the point of infection, this disparity is still more strikingly illustrated, due mainly to the very toxic character of the poison produced by the tetanus bacilli.

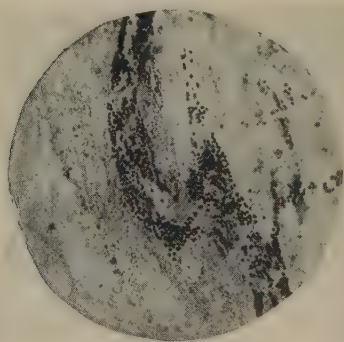


FIG. 23.—Large masses of pneumococci in the blood vessels of a rabbit dying of severe pneumococcus septicemia. BULL.

Neither is the size of the focus related to the rapidity with which a cure may be effected. A very small or localized infection may be very difficult to cure. This may be due to the slight amount of irritation it causes and the consequent fact that it arouses very little general body reaction, (See Vaccines, p. 186), or to the completeness with which the lesion has been closed off from the rest of the body. In tuberculosis, for example, the tubercle wall which is formed around the lesion not only protects the body from the organisms, but protects the organisms in that lesion from the blood anti-substances which might destroy them. Similar conditions obtain in meningitis, where the organisms in the meninges of the spinal canal are very slowly affected by the anti-substance of the blood, because of the relatively meagre blood supply of that area.

Preferred Method of Entrance.—Usually infection occurs only when bacteria enter by certain preferred routes or channels. For some organisms the “channel of infection” is very definitely fixed. Typhoid, for example, must begin its development in the alimentary canal and one usually contracts typhoid by swallowing the typhoid organism in infected food or water, there being little chance of contracting typhoid infection in any other way, such as through the air. (Recently the tonsils have been shown to be possible, though less probable portals of entry, than the more common path, the intestinal membranes.)

Sometimes the preferred method of entry seems wholly unrelated to the final site of the organisms. This is strikingly illustrated by hookworm, in which 90 per cent. of the infection is through the skin, and the hookworms make a circuitous route through the flesh to lymph vessels, and by the thoracic duct to the heart and to the lungs; from the lungs they are coughed up into the throat and being swallowed make their way to the intestines, their real habitat.

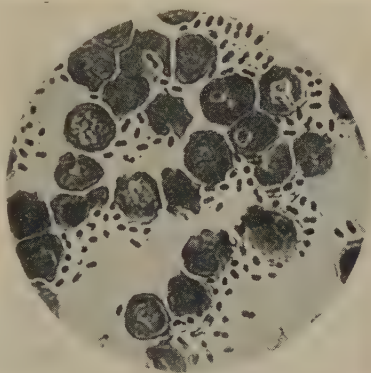


FIG. 24.—Blood smear showing the invasion of the blood by pneumococcus organisms, (*Diplococcus pneumoniae*). The characteristic capsules are very evident here. Williams, Brown and Earle.

More often in other infections it is a question of being carried from the port of entry by the circulating blood until a susceptible area is found, where the organisms may continue to develop after they have disappeared from the conveying blood. Despite these vagaries, the initial point of entrance is very important in determining whether or not infection shall occur.

Experiments may be performed to illustrate preferred methods of entry. As described by Park, if virulent streptococcus (Fig. 28), typhoid, and diphtheria organisms respectively are rubbed into abrasions of the skin of laboratory animals the typhoid produces no lesion, and the diphtheria but a very minute

infected area, but the streptococcus may produce severe cell destruction or even fatal blood poisoning (Fig. 29). When placed on a similar area of the throat, the typhoid is again harmless, the diphtheria produces a typical inflammation and characteristic toxic poisoning (p. 87), and the streptococcus may cause an infection, or even blood poisoning. If the three kinds of organisms are passed into the intestines, it is the typhoid organism which develops, while here the streptococcus and diphtheria are harmless.

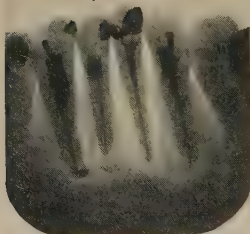


FIG. 25.—X-ray photograph of a tooth showing a definite disintegration area at the apex of the tooth. Such abscesses often furnish an illustration of very untoward effects from a very small focus of lesion.



FIG. 26.—A pure culture of *Streptococcus viridans*, taken from a root apex of a tooth; this tooth had a good root-filling and the X-ray examination did not show decay.
MACHAT, *Dental Cosmos*.

As stated on p. 36, bacteria that enter the body may fail to develop even when the growth conditions are apparently favorable. When such organisms as streptococci or staphylococci which can grow in the body fail to cause infection, after gaining entrance through a cut or bruise, or cause but a slight temporary infection, what is the explanation? It is because there was in the blood of the body more than a sufficient number of white corpuscles to take care of the invaders, and the circulating blood finally accumulated in that region enough white corpuscles to dispose of them; or else the liquid antibodies in the blood dissolved or otherwise affected the bacteria, as will be described in the next chapter.

Number and Virulence Related to Resistance.—There is, of course, a limit to the counteracting effect of these protective agencies of the normal healthy body. The minimum number of bacteria which is necessary to run the gauntlet of these guarding agencies explains such statements as “ten tuberculosis bacteria are necessary to inoculate a rabbit.”

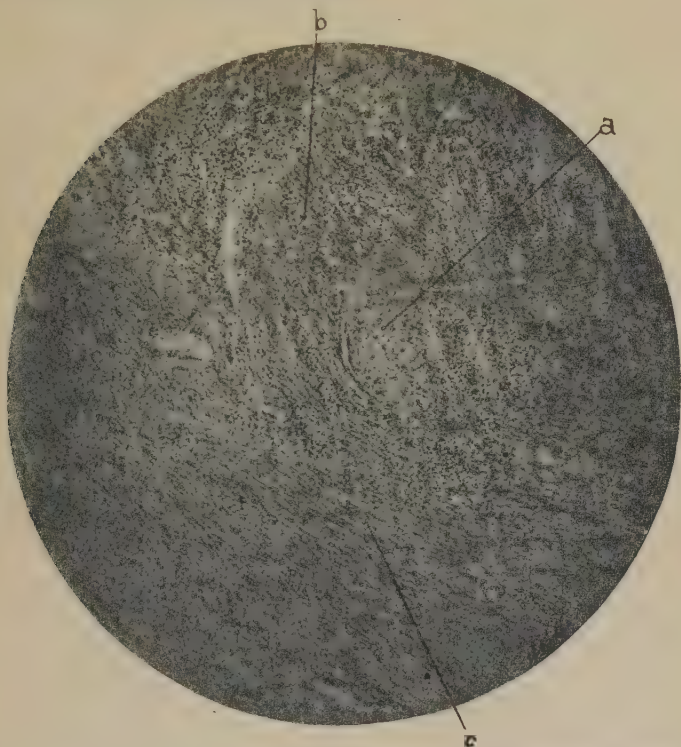


FIG. 27.—Disintegration due to cancer; *a*, cancer nest-cells with extensive inflammatory infiltration of muscle bundles, *b*. KELLY, *Johns Hopkins Bulletin*.

This minimum number depends on the kind of organism as well as the natural resistance of the individual. The minimum may be very low, indeed; for anthrax (Fig. 30), it has been stated that one organism is enough to cause infection in a susceptible animal (See p. 36).

In other cases the number necessary for infection is very great,

and the type of infection may vary with the number inoculated; *e.g.*, when a few streptococci pathogenic to man are inoculated into a rabbit, they may cause no effect at all, and a few million may cause merely local abscesses, while a hundred million will usually cause septicemia (general blood poisoning).

The number necessary for infection varies also with the strength and virility of the organisms (See p. 58). If a strain of pneumococcus organisms is cultivated in two different ways—



FIG. 28.—A long-chained streptococcus, *Streptococcus pyogenes*; notice the relationship of many of the organisms in the chain, often present in this genus. Williams, Brown and Earle.

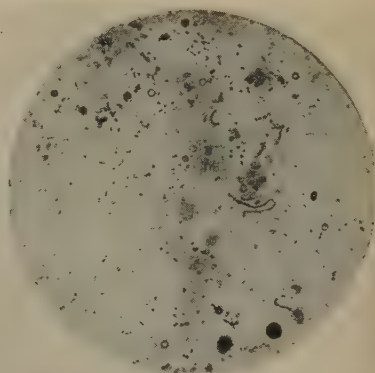


FIG. 29.—Streptococcus growth in peritoneal fluid freshly drawn from a fatal case of peritonitis. Note the heavy growth of bacteria.

(1) in a series of tubes of artificial media, such as serum-agar, and (2) in a series of living mice—the organisms from the last mouse will be much more powerful than the organisms from the last serum-agar tube. It may take one-tenth of a cubic centimeter of the agar strain to kill a mouse, but it may take only .000001 of a cubic centimeter or even less of the mouse strain to bring about the same result.

There is also a great initial difference even in freshly isolated strains of a given kind of bacteria. One culture is often fifty or more times as virulent as another; experiments have shown that a less virulent diphtheria culture may require .1 c.c. for a fatal dose, but .022 of a c.c. of a virulent culture of diphtheria may be equally fatal.

Infection and Immunity.—A slight infection, such as a tiny abscess around a splinter, may develop even though the body as a whole really has sufficient protective or immunity-yielding substances, because such substances have not yet been marshalled to that area in sufficient quantities. Serious infections, however, occur when, because of the low level of normal protective agencies, or the high virulence of the invasive bacteria, or both of these conditions, the body is for the time being relatively unprotected. More white corpuscles have to be manufactured and special new antistances have to be formed in the body before an individual so infected can overcome the invading organisms; in doing so he develops an active or "acquired" immunity to such organisms. This definite or specific immunity is the subject of the following chapter.

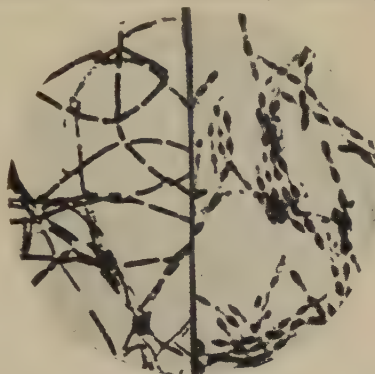


FIG. 30.—Anthrax (*Bacillus anthracis*).
Vegetative form on the left, spores on the right. Williams, Brown and Earle.

STUDY SUGGESTIONS

1. List and describe the various effects of micro-organisms upon the human body.
2. Consult texts describing specific diseases to find specific illustrations of each of the various effects of bacteria or protozoa upon the human tissues.
3. Find in a text book of bacteriology a comparison (bulk or weight) of the killing strength of a strong drug such as strychnine and a given preparation of tetanus, botulinus or other bacterial toxins.
4. Explain why fever may be considered a symptom rather than the disease itself.
5. Describe the passive body defences against bacterial invasion.
6. Find an illustration emphasizing the importance of cell vigor in resisting disease.
7. Illustrate "preferred methods of entrance for diphtheria, for typhoid, or for tetanus.
8. Copy the skeleton outline of the contents of this chapter (p. 17) on a large sheet of paper and elaborate each of the sub-topics so as to cover the subject matter given in the chapter.

9. It has been demonstrated that such conditions as shock, thirst, starvation and collapse can cause disintegration of cell tissue. Which of the results of bacterial action would the accumulation of such disintegration material parallel or resemble? How would such conditions affect the subject's resistance to bacterial infection?
10. An investigator reports that one-ten-billionth of a loop of some cultures of the hemorrhagic septicemia organism kills a rabbit in 24 hours, at which time bacteria can be demonstrated on every drop of blood or body fluid examined. Can you express definitely the "minimal lethal dose" of such an organism?
11. It has been calculated that some bacteria can divide into two new ones every twenty minutes. If such multiplication is unchecked by body reactions, etc., how many organisms could be formed in 24 hours from a single initial bacterium?
12. Look up, in U. S. Public Health-Service Reprint 436, the mode of transmission of the diseases described there. In how many of the diseases listed are there evidently "preferred methods of entrance?" In how many must the causal organism be inserted directly into the tissues (insect bite, etc.)?
13. The mode of transmission of leprosy is described as "close, intimate and prolonged contact with infected individuals." Can you explain this on the basis of the size of the minimal lethal dose, variations in individual resistance, or both?
14. Stillman (*Health News*, Sept. 1921) says that "careful histories of patients with lobar pneumonia show that about 40 per cent. of the cases give a history of coryza or other mild infection of the respiratory tract preceding the onset of pneumonia." What two reasons—one direct and one indirect—can be advanced to explain the importance of such mild or unrecognized infections?

CHAPTER II

ACTIVE IMMUNITY *

Active Immunity	{	Sources or origin of protective substances	
		Types of reaction against	<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 2em; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle;"> Toxin Bacteria </div> </div> <div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> <div style="font-size: 2em; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle;"> Acting alone Acting with white cor- puscles </div> </div>
		Nature of reactions	<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 2em; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle;"> Chemical in character Graphic illustrations </div> </div>
		Disease and the balance of bacterial action and body reaction	<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 2em; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle;"> In recovery from disease In death Length of immune period Variations in bacteria virulence </div> </div>
		Types of active immunity (Racial, natural etc.)	
		Substances used to induce immunity (Introductory to vaccines)	<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 2em; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle;"> Live organisms Dead organisms Toxins </div> </div>

It is said that over 1900 years ago Mithridates the Great secured immunity against certain poisons by subjecting himself to gradually increased doses of those poisons. The "Mithridatic antidote," according to Pliny, was composed of sixty-four ingredients, some of them used in infinitesimal amounts, as small as one-sixtieth part of a drachm, which is about one-five-hundredth of an ounce (apothecaries' weight). With these substances he "mixed the blood of Pontic ducks, because they lived on poisons." Despite this early essay in immunity production it is only in the last generation, except for the preventive methods in relation to smallpox, that any practical methods of acquiring immunity to disease have been developed.

* This chapter, with the following, Chapter III, forms a general introduction to the subjects of active and passive immunity, the mechanism of which is discussed much more in detail in the subsequent chapters on antitoxins, vaccines, etc.

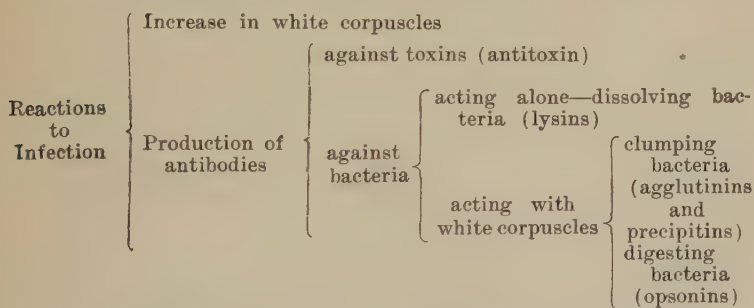
Reactions to Bacterial Poisons.—As shown in the preceding chapter, bacterial poisons irritate or destroy one or more of the body tissues. The normal individual responds to such irritation, accumulating in his blood and lymph (1) an increased number of white corpuscles, and (2) special antistances, or *antibodies* as they are commonly called, formless substances which are contained in the liquid part of the blood and lymph.

Sources of Protective Substances.—The white blood corpuscles are formed in the bone marrow and the lymph nodes or glands. The antibodies are probably formed mainly in these same places, and in the spleen as well; in fact, many believe there is no evidence to indicate that these antibodies may not be formed anywhere in the body. In some cases they are evidently formed quite locally, by the tissues directly affected by the disease organisms. This it is thought may explain why in a prolonged attack of boils the latest boil always breaks out in a new spot, or why erysipelas spreads only to new regions, avoiding previously inflamed or affected areas; it is quite evident in these cases that the local immunity is greater than any general body immunity.

Two Types of Reaction.—It has already been shown (p. 18) that the effects of bacteria are mainly due to two causes: (1) to the toxins they form, and (2) to the poisons formed from the bacterial cells themselves as they are disintegrated in our bodies. It is to be expected, therefore, that the body will respond or react in two different ways:—(1) to neutralize or destroy the bacterial toxins and (2) to destroy the bacteria themselves. This last response would be the most effective, of course; for it would be final; with the destruction of the bacteria would cease their production of toxins. But in some diseases, toxins are excreted very rapidly. Since toxins are injurious in very small amounts, (1 c.c. of tetanus toxin can kill 75,000 guinea pigs), bacteria may give out dangerous amounts of such toxins very early in the disease—before the bacteria begin to disintegrate sufficiently to stimulate the body to react against their disintegration products, *i.e.*, the split-protein poisons. (See p. 21.) The first response mentioned above, producing antibodies against the toxin, is therefore a necessary one when toxin-producing bacteria cause the disease, as in diphtheria and tetanus. However, even in these diseases, reactions inimical to the bacteria themselves are also

essential. And since the destruction of these bacteria involves more or less irritation due to their split-protein disintegration products, it is essential that the bacteria be destroyed as promptly as possible. The more prompt such body reactions, the fewer bacteria there are to be thus destroyed, and the less split-proteins are set free in the body. The body responds to all bacterial infections are thought to be "essentially antibacterial in character: that is, by the development of specific antibacterial antibodies and their action, as well as by the activity of the phagocytic cells."

Tabulation of Reactions to Bacteria.—The antibodies vary in their way of attacking the bacteria or neutralizing their products. Some kill the bacteria directly, others help the white corpuscles destroy them; and, as just described in the preceding paragraph, other antibodies are effective only against the toxins. The following tabulation of these reactions may be helpful in visualizing these distinctions.



In most diseases, probably more than one reaction occurs, though one is always much more prominent than the rest. In diphtheria, for example, there is a lysin formed which helps kill off the bacteria, but it is so much less important than the other antibodies, including antitoxin, that the blood of animals immune to diphtheria may fail to show any lysin at all when tested for lysin. The relative unimportance of the lysins in diphtheria is also shown by the fact that in experimental animals inoculated with living diphtheria organisms, there may be recovered from the scabs at the site of the injection live diphtheria bacteria after the animals have become immune to diphtheria; bacterial lysins,

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therefore, cannot be the most important factor in diphtheria immunity. In discussing any particular disease, it is, therefore common to emphasize one reaction, antitoxins for example in diphtheria. That means only that this is apparently the most important of the reactions, or the one most readily measured or determined, and does not necessarily imply that it is the only reaction made against the disease.

Theories of Cell Relations to Foods and Toxins.—Toxins injure certain cells because they have the power of chemically uniting or combining with these cells; they lack such chemical attractions or affinity with other cells and so those cells are not directly affected. The difference in chemical affinity explains why it is mainly nerve tissue that is destroyed in lockjaw (or tetanus) and infantile paralysis; diphtheria toxins, on the other hand, are much more catholic in their tastes and, as already described (p. 20), combine with many different types of cells.

This chemical affinity is doubtless the same type of selective affinity that influences the cell in its selection and utilization of food materials. One food element can be combined with a given cell and utilized; a second food element cannot combine and never enters into intimate association with that cell. It is doubtless just such selective differences that make possible the differentiation of the cells into such specialized types as muscle cells, gland cells and nerve cells.

Since toxins are more or less protein in character, it is conceivable that a given toxin may have the same chemical affinity as a given food protein. If then, such a toxin combined with a cell, it would have two undesirable or injurious effects upon the cell: (1) it would prevent the normal combination with the special food element whose place it had usurped; and (2) its chemically irritating poisons would be brought into intimate association with the cell, irritating or destroying it.

Graphic Illustrations of Chemical Reactions.—While, as described above, these combinations and processes are chemical ones, so many people are eye-minded that students often find it helpful to have these combinations of food and toxins with the cell represented pictorially or diagrammatically. Some modifications of the diagrams in common use are given below, with the

warning that these representations are purely imaginary, and that the reader must remember that the combinations and activities they illustrate are really chemical, and that such static morphological structures must not be allowed to supplant in our minds dynamic chemical processes. Recently something of a revulsion against this "side-chain theory," as it is called, has been evident; and the student is therefore encouraged to emphasize constantly the actual affinities and activities themselves, remembering that the chemical processes these illustrations depict are really much more wonderful and mysterious than the complicated system of diagrams people have elaborated to help explain them.

Cell and Food Relations.—The chemical affinities of the cell for food materials may be partly illustrated by the diagram (Fig. 31) in which are shown a cell and above it five types of food materials; these are lettered

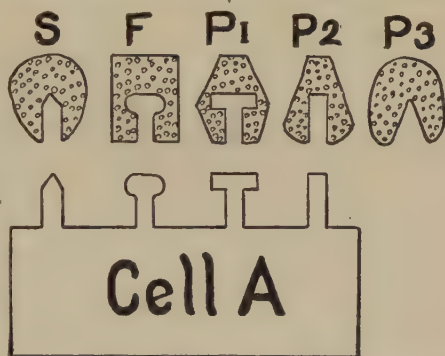


FIG. 31.—A diagrammatic representation of a given cell's power to utilize four of the five food particles shown above it.

to indicate hypothetical molecules of a sugar (S), a fat (F), and three different proteins (P1, P2, and P3). Note that the following illustration (Fig. 32) shows one food (P3) not utilizable by Cell A. This food, P3, may be utilized by another cell, as illustrated by cell B, which may in turn lack the power to use one or more of the foods usable by cell A.

Suppose that a toxin particle by virtue of its protein character, resembles P1 in its chemical affinity—in its attachment structure (Fig. 33).

It could then attach itself to the cell just as P1 would. The cell would suffer in the two ways already mentioned (p. 50): (1) It would lose the food that might satisfy the combination, and (2) it would be irritated or damaged by the chemical action of the toxin. Instead of cell action on the usual food material, we might have toxin action on the cell.

When a cell is well nourished and vigorous, it gains the power of caring for or utilizing more food material. When food material is made over into cell substance the cell can be pictured as a stronger or even larger cell, and, therefore, having increased affinities for the same kind of food substances. The more it utilizes, the more it can use. This may be illustrated by an increase in the attachment points or receptors, as they are called, showing that when food occupies all the receptors on a cell (Fig. 34) it is then stimulated to form more receptors to which food may be attached (Fig. 35).

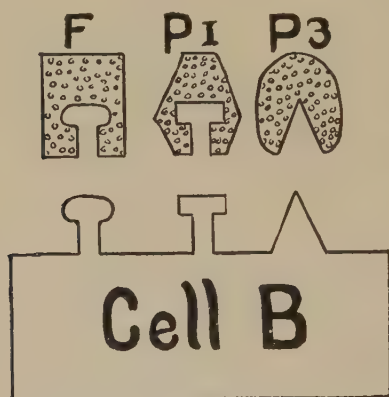


FIG. 32.—Cell B, having the power to use P 3, which Cell A lacked.

food particles are the stimulating factors; but instead of keeping these chemically-combining factors as parts of the cell, they are thrust away from the cell, into the surrounding blood or lymph, (every living cell being bathed by blood or lymph on one or more sides). Thus these receptors or combining factors meet the toxins before they reach the cells and combine with them while they are still in the blood or lymph; the living cells are thus protected by outlying guards of the substances they have formed, the extruded or thrust-off receptors (Fig. 37). When, as just described, such receptors neutralize or combine with a toxin, they are called antitoxins. Similar substances combining with bacteria themselves might be given a general descriptive name such as antibacterins to indicate that they act on bacteria cells, not merely on their products, the toxins. No such general

Cell and Toxic Relations.—If toxins were present in the neighborhood of such cells *e.g.*, in the circulating blood, these extra resources if toxins do begin to be a disadvantage, unless they could be satisfied more promptly by food than by toxins.

However, the body is not wholly without protective resources if toxins do begin to combine with the cell (Fig. 36). The cells form extra receptors just as when

term is in common use, however, and the substances formed against the bacteria are given special names depending upon the way they affect the bacteria: *lysins*, or *bacteriolysins*, if they dissolve bacteria; *agglutinins*, if they clump the bacteria together, etc. (See p. 49.)

The cell-food and cell-toxin combinations described in the preceding paragraph are the simplest of the series of the combinations we shall discuss in this volume. But even these may seem impossible and fantastic. That there are, however, such relationships, and that the food-attaching and the toxin-uniting powers are somewhat similar and interchangeable is indicated by several interesting bits of evidence, among which are the following variations in susceptibility to disease.

When individuals are in a well nourished condition they are much less susceptible to disease. When even temporarily less well nourished they may come down with a disease to which they had reason to consider themselves immune, *e.g.*, nurses and physicians through overwork or after a sudden nervous shock often succumb to epidemics they have successfully faced for weeks or months. A lack of properly digested food, whether due to actual lack of food, or incomplete digestion of a plentiful diet, related to overwork, fear, worry or any type of nervous strain, may leave unoccupied or unsatisfied the cell affinities or combinations by which toxins or other bacterial products may unite with the cell. This same idea underlies such common expressions as "If you are afraid of such and such a disease you'll get it," or "She worried herself into it," or "She broke down nursing the rest of the family and then she took it."



FIG. 33.—Toxin resembling such a food as *P I* in the cell-combining power.

Disease and the Balance between Bacterial Action and Body Response.—In disease, the bacteria multiply rapidly until their toxins or disintegration products stimulate the tissues capable of definitely reacting against them to form extra white corpuscles or special antibodies, or both. The bacterial growth may be represented by the line *bc* in Figure 38, *b* representing the entrance of the bacteria into the body. Finally the body responds, beginning to develop antibodies at *a*; and if the indi-

vidual reacts vigorously, the increase in antibodies may be as rapid as represented by the average rise of the dotted line *a-c*. The symptoms or set of symptoms evident to the patient or doctor may appear before or after the time the protective internal reaction begins. They are indicated afterward here, by *s-t* to *s* then represents what is called the incubation period which may range from two or three days to two or three weeks, or, rarely even longer, as in rabies. (See p. 56.) At the crisis *c*, the patient's reaction as shown here is balancing the bacterial action. This reaction, however, continues for some time as indicated by the extension of the dotted line beyond *c* to *a*. Finally, however, the overproduction lessens, for the bacterial

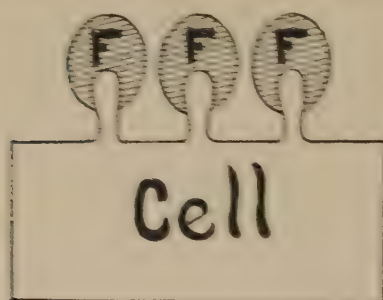


FIG. 14.—Diagrammatic representation of cell and food consumption.

irritation is decreasing rapidly as shown by the decline in bacteria or toxin production, the line *c-e*, and finally at *e* the bacteria and their poisons have all been eliminated from the body. The antibodies are not eliminated so rapidly, though they, too, gradually disappear from the blood; in the diagram above, their disappearance is indicated at *i*.

and the patient is then no longer immune to that disease.

If the patient described above had responded less vigorously the lines starting from *b* and *a* might not have met as soon, or, as shown in Fig. 15, they might not have met at all. In that case the prolonged bacterial irritation indicated by the continuation of the *b-a* line would finally cause the death of the patient. There would be no "turning point in the disease."

This whole situation has been well described by Park: "It is the unaided skirmish between the two [invading germ and invaded subject] which determines whether or not a foothold shall be gained upon the body of the subject and an infection thus established; and it is the balance between them which decides the eventual outcome of recovery or death."

Length of the Immune Period.—The length of the immune

period (*e* to *i* in Figure 38 following) depends upon how vigorously the individual reacted, how much excess antibody material was made, and upon how rapidly it was eliminated from the body. After smallpox, immunity usually lasts throughout life; after vaccination against smallpox it usually lasts several years, and in one known case it was demonstrated by Jenner that smallpox vaccination gave immunity lasting at least fifty years. In such a case the line *o-i* in our diagram would be a very long line indeed. In some diseases immunity lasts but a short time; that is noticeably true in common colds, where one sometimes contracts a fresh cold before the last one is over. In such cases the line *o-i* would almost coincide with the line *c-e*.

The immune period, as intimated above, varies greatly also with the individual. A reliable authority some time ago reported a child of eleven who had had several attacks of three diseases, each of which ordinarily yields an immunity lasting at least several years; the record was diphtheria

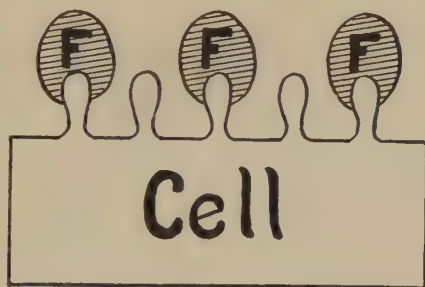


FIG. 35.—Diagrammatic representation of the increased size and vigor of a food-satisfied cell, and its consequent increased power of utilizing more food material.

three times, measles three times, and scarlet fever twice. In each attack this child had evidently made very little overproduction of immune substances, or else she had eliminated such immune substances with unusual rapidity. In either case her line, *o-i*, in the diagram (Fig. 38) corresponds very closely to the line *c-e*.

Elimination of Antibodies.—The rate of disappearance of antibodies as above described varies with the disease, and with the individual. How such elimination takes place is not definitely known; the liver and spleen and also the urine have been suggested as possibly connected with the elimination of such substances.

Antibodies as Aids in Diagnosing Disease.—As long as antibodies remain in the blood in any considerable amount their presence there may be detected by bacteriological methods, as described later for each type of antibody in its respective chapter.

These antibodies are specific. That is, the antitoxin for diphtheria neutralizes diphtheria toxin and not other toxins, such as botulinus toxin; the opsonins for staphylococcus organisms aid white corpuscles in disposing of staphylococcus organisms and do not help in gonorrheal or tubercular infections. The occurrence of such antibodies is therefore taken as an indication that the individual has or has had the related disease.

Variations in Virulence.—Among the organisms which can grow in our tissues some are much less irritating than others (See p. 211). This difference is partly accounted for by attributing to some bacteria the power to form definite substances (aggressins, p. 150), which help them invade the tissues, while other bacteria evidently lack such power. Lessened virulence may also be explained by considering that such organisms form very small

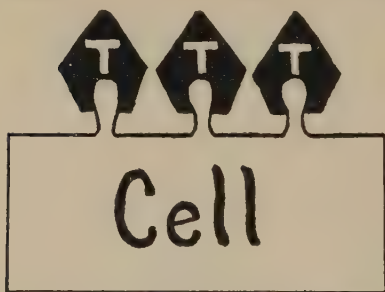


FIG. 36.—Diagrammatic representation of toxins (T) combining with the cell.

amounts of poisons or very mild ones, or that they are very like the cells of our own bodies in their metabolic processes, and that their action, therefore, is not very irritating to us. Such organisms would not arouse decided or marked reactions, and might continue to develop in the body for years, even throughout life,

without at any time causing a condition serious enough to produce death, as is sometimes true of tuberculosis. Such bacteria have been spoken of as “successful germs.” They are so adapted to body conditions, that they develop without arousing violent antagonisms and also without destroying the host on which they depend for their existence. In carriers, such as typhoid carriers, where typhoid organisms have been known to persist in the intestinal membranes of an individual for over 45 years, there is a similar adaptation between the organism and the host. Such adaptation, as might be expected, is not uncommon in protozoan diseases, for the causal micro-organisms are animal cells and naturally more like our human cells in general metabolism. Syphilis and malaria are common examples of such “successful germs” among protozoa.

Spurring Body Reactions.—In certain more or less chronic

diseases such as the above, treatment is sometimes based on the theory that an increased or heavier dose of the slightly irritating substances will "jog" the system into a more pronounced reaction with a consequent overproduction of antibodies and thus give the individual a definite immunity—at least for a short period. Boils sometimes occur in a prolonged series extending over months, and treatment on this basis—with injections of large numbers of dead bacteria of the kind which are found in the boils—has seemed helpful in many cases, being followed by an immune period of several months, or complete recovery. (See Vaccines.)

Variation in a Given Species.—A given disease organism does not always have the same degree of virulence. (See also p. 44.) We recognize this when we say "Just a light attack of measles, the kind all the children are having," or "Influenza seems to be of a particularly virulent type this winter."

Causes of Variation.

—Organisms are affected by such outside conditions as cold and lack of moisture in the atmosphere. (See also p. 37.) Organisms weakened or attenuated by such unfavorable conditions may, on entering the body, multiply less rapidly and produce less injurious and irritating conditions. But probably the conditions met by the organisms in the last host or individual often have even more to do with the vigor or strength of the organisms eventually passed on to the next individual. It is easy to understand that a vigorous reaction on the part of an individual having the disease might not only so affect or weaken the organisms while in his body that he had a mild attack of the disease under consideration, but might so affect the organisms that when such weakened organisms were passed on to the next individual, they would cause only a mild or light form of the disease. An attack of scarlet fever, for example, contracted from such a mild case is more likely to be a mild attack than one contracted from a severe case.

The above does not always follow, of course. A severe case

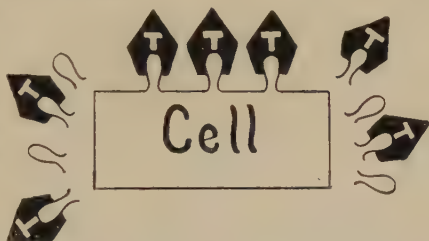


FIG. 37.—The toxin has stimulated the cell to produce other receptors, which neutralize the arriving toxins and prevent further toxin-cell combinations.

may be contracted from a mild case. The most likely explanation of this is that the person having the severe case had very little individual resistance, and that even the mild organisms passed on to him made relatively greater inroads than with the first more vigorous individual who had the mild case. (See also p. 59.)

The severity of a disease may, therefore, be expressed as a balance between the resistance of the individual and the virulence of the organisms. This may be graphically represented by the following equation: Resistance—Virulence=Body Condition. If the virulence is greater than the individual's resistance we have a negative result, a state of disease. The degree to which the

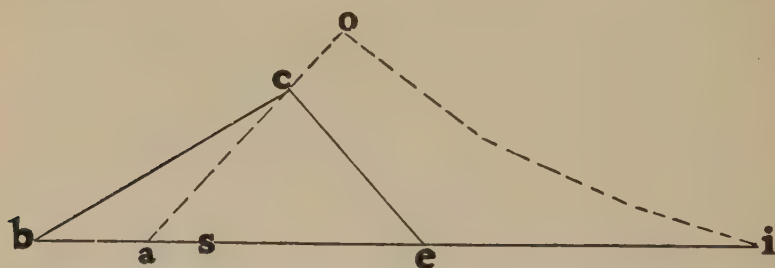


FIG. 38.—Diagrammatic curves showing antibody production and immunity with relation to disease. As described in the text, *b* indicates the entrance of the bacteria; *a*, the beginning of the formation of antibodies, with an increasing rate of production as shown by the broken line (*a-o-c*), where bacterial production (*b-c*) and antibody production (*a-c*) meet might be termed the crisis; at *c*, bacterial production falls off, with a final elimination of the organisms at *e*; antibody overproduction, however, continues to *o*, and the immune condition of the patient persists until these too disappear, *e* to *i* representing the immune period.

resistance overbalances or exceeds the virulence determines the health of the individual.

Virulence Increased by Animal Passage.—The virulence of an organism may be increased by growing it in a “favorable animal” or in a series of such animals. For example, the virulence of rabies organisms may be greatly increased by so “passing” them through rabbits. If organisms from the first rabbit are inoculated into a second, and then from the second into the third, very virulent rabies organisms may be secured from the third rabbit. Many other examples could be given of such increase in virulence by animal passage: notably, streptococcus and pneumococcus organisms in rabbits and glanders and diphtheria in guinea pigs. The selection of the animal is important; *e.g.*,

passage through monkeys decreases the virulence of the rabies organism, while, as described, passage through rabbits increases it.

The accidental transfer of organisms from person to person may produce organisms of increased virulence. How far the severe forms or types of organisms characterizing certain epidemics, such as the infantile paralysis epidemic of 1916, are due to more favorable external conditions, and how far to such a chain of perpetuating individuals we cannot say.

It may not be as simple as we have just implied. Unfavorable atmospheric conditions may decrease the number of living organisms and therefore one's chance of infection; but the organisms which survive, survive because they *are* the most vigorous and resistant, and the cases subsequently caused by them

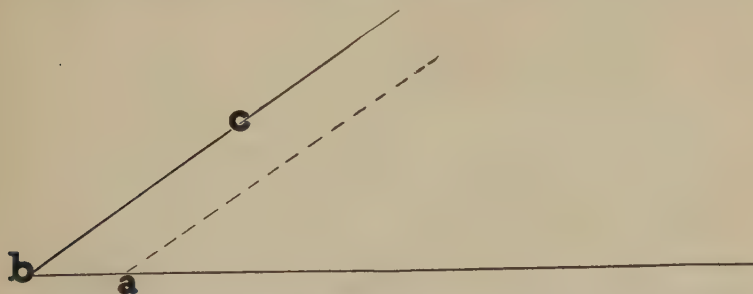


FIG. 39.—When the antibody responses do not exceed the irritating or toxic effect of the bacteria, the antibody line beginning at *a* cannot cross the bacterial line at *c*, as in the previous illustration, and there is no marked crisis change, and no recovery followed by an immune period.

may be more severe for that very reason. Then, too, it is quite possible that where “animal passage” causes an increase in virulence, it may not be simply because the animal is susceptible, but because the animal is resistant, and while the weaker organisms are killed off, only the stronger and more resistant ones survive, and so a race of more virulent organisms is bred and passed on.

Relation of Virulence to the Incubation Period.—While the incubation period of most diseases is quite definitely known, it is usually expressed with a margin of one to three days, *e.g.*, yellow fever three to five or six days. This range is more or less explained by such variations as the size of the causal dose, the initial number of organisms infecting the individual. Other important factors are the individual's degree of resistance, and the virulence of the organism. A shortened incubation period, there-

fore, is often characteristic of the more severe attacks of any given disease.

A short incubation period is similarly characteristic of some of the most dreaded diseases, *e.g.*, yellow fever three to five days, gonorrhea one to eight days, and diphtheria two to six days. This is not an invariable rule, however; there are many exceptions, such as smallpox with a two-week incubation period, and rabies with an incubation period varying from two weeks to several months.

Natural Immunity.—Certain types of variation in susceptibility to disease are usually discussed under such headings as natural immunity, racial immunity, and resistance due to age. These variations include such differences as the relative prevalence of yellow fever in the white and negro residents of a given region, or the proportion of diphtheria cases in the adult and infant population of a community. For some diseases, this reputed difference in susceptibility may be supported by blood tests, such as the Schick test by which the individual susceptibility to diphtheria may be determined (See p. 100).

Some of these so-called differences in susceptibility are doubtless due to differences in the opportunities for infection or to differences in resistance dependent upon the hygienic character of the surroundings. It is very evident that when two races so compared represent different economic levels, *racial* differences in susceptibility can not be demonstrated unless both races have an equally satisfactory environment with regard to all essential details—ventilation, methods of waste disposal and other phases of sanitation, as well as the character and quantity of the food supply and freedom from over-exposure and over-exertion.

Occasionally, the advantage may seem to be with the race of lower economic status, as in malaria or other tropical diseases. This is not because environmental conditions are unimportant, but because selection through many generations has eliminated in the more resistant race the individuals specifically less resistant to any given disease, and bred up a race tolerant or even immune to the disease in question.

Racial Immunity.—Variations in susceptibility to disease have been noted in different breeds of the same kind of animal, such as sheep, and in the different human races. A well-known

illustration of such racial differences is the immunity of Algerian sheep to anthrax (Fig. 40) while ordinary sheep are susceptible. Similarly, pigeons are immune to anthrax (Fig. 30) while most birds are susceptible. Field mice are very susceptible to glanders, while white mice are immune. Among insects, mosquitoes afford similar parallels; the genus *Anopheles* being the common host of the malarial organism, while the genus *Culex* rarely serves in this



FIG. 40—*B. anthracis* with capsules from liver of guinea pig. ($\times 1500$)
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way. For man, similar variations are found in comparing the negro and white races; the negro, for example, being less susceptible to yellow fever and more susceptible to tuberculosis and pneumonia.

Natural Immunity.—Individuals differ in their susceptibility to disease. These differences may be temporary only, such as the variations a given individual might show depending upon his physical condition. As might be expected susceptibility increases with fatigue, over-exertion, worry, undue exposure to chill, overheated or badly ventilated rooms, retention of body

excretions (*e.g.*, feces), the accumulated effects of a previous disease, and with alcoholism.

Individuals often claim a natural immunity for what is really an acquired immunity to a given disease. Such immunity may be due to what is practically a previous but very slight attack of the disease—to the earlier unrecognized entrance of a small number of the organisms in question, through such membranes as the respiratory or intestinal tract, which stimulated the production of sufficient protective substances. Dysentery and typhoid may be cited as illustrations of such a condition, appropriate antibodies (agglutinins) having been demonstrated in the blood of individuals who are known to be immune but for whom we have no records of the disease in question.

Besides these variations there are differences somewhat more predictable, which are generally considered to be related to the age, race, or species of the individual. There are, for example, certain diseases commonly classed as children's diseases, partly because children are more susceptible to those diseases than adults, and partly because, since most adults have had such widely spread diseases when children, they are afterward more or less immune to them, and therefore most of the cases in any period occur among the children. Ringworm and thrush are infections to which children are very susceptible. As contrasts one might cite diphtheria and typhoid as diseases to which very young children seem immune (See p. 102); such "natural immunity" may be a transferred immunity—a passive immunity due to substances transferred from the mother before birth—or, less often, with the milk.

As these gradually disappear, the child becomes more susceptible; the most susceptible period for diphtheria, for example, follows very closely upon the period of inherited immunity,—between the ages of one and four years.

The immunity that sometimes comes with age cannot always be explained as due to recovery from an attack of the disease. Many individual instances are doubtless due to recovery from mild and unsuspected cases of the respective disease; for example, what is thought to be an attack of dysentery may be a typical typhoid fever, yielding the usual evidences of immunity: agglutinins in the blood, and resistance to later typhoid infections. The phe-

nomenon of increased resistance with age, is too common to be explained on this basis, however, and is generally ascribed to the invasion of a few of the micro-organisms through the mucous membranes, such as the tonsils, nasal membranes, or intestinal linings, and the consequent arousing of the usual immunity responses in effective though relatively low amounts.

There is still a great deal that is unexplained in this question of natural immunity; and in this connection we must not lose sight of the fact that the *chemical* conditions or balance of the body cells or tissues are of fundamental importance. Slight differences in chemical structure or composition favor or resist the combining effects of the various bacterial products. To such unascertained differences we must attribute differences in variety resistance, such as the resistance shown by certain strains of wheat to rust, certain species of sheep to anthrax, or different races of man to yellow fever and tuberculosis.

Practical Methods of Inducing Active Immunity.—(For a more detailed discussion of this phase of active immunity see Vaccines.) Since, when one recovers from a disease, he usually has secured immunity to that disease for a period of time, it would naturally occur to any one interested in the health of a people that it would be an advantage to give well people a mild attack of a given epidemic disease and thus prevent serious cases. Several of the ancient Asiatic peoples recognized this, and practiced it with smallpox. Indeed, in some parts of China to-day the natives put pus from the eruptions of mild smallpox cases on cotton or wool plugs and insert them in the nose. The cases thus caused are usually light cases and give immunity for life. But as shown earlier in this chapter (p.58) severe cases may result from light ones. One can never tell just how virulent such organisms are, nor just how much resistance the next individual will have. It will be much safer, therefore, if some definite attempt is made to weaken the organisms before they are inoculated.

Several different methods may be used for this: (1) growing the organisms in a less favorable animal; (2) growing them under unfavorable laboratory conditions, such as too high a temperature or in unfavorable food materials; or, (3) subjecting them after growth to unfavorable conditions, such as drying, chilling, or the addition of chemicals. Such weakened organisms

are very unlikely to cause very serious attacks of their respective diseases. In such diseases as typhoid, however, where bacteria may so accustom themselves to the body, that they continue to live and multiply in the intestine after the individual has apparently recovered, there would be a risk in giving *live* organisms, no matter how weakened, for the individual might thereby become a "carrier" for that disease. In diseases where the infection may be transferred by the body excretions (nasal excretions, feces, etc.), it is, therefore, undesirable to give any individual live organisms, no matter how weakened, if a better plan can be found.

Fortunately, in diseases like typhoid (Fig. 41), good results

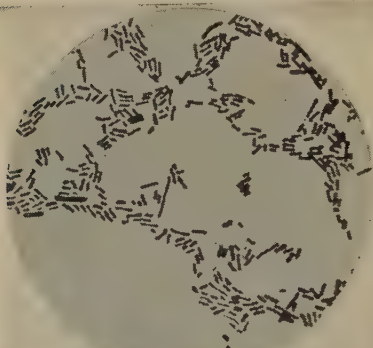


FIG. 41.—Broth culture of *Bacterium typhosum*, the typhoid organism. WILLIAMS, BROWN and EARLE.

are obtained if the bacteria are first actually killed. The bacteria are killed by heat, by chemicals such as carbolic acid, or less often by grinding. Broth or agar cultures of the bacteria are usually used in this work. This material contains the same irritating substances that would follow the development of live bacteria in the body: toxins, poisonous split-proteins (See p. 173), and the body is stimulated

therefore, to produce the necessary antisubstances.

In a few diseases, it is not necessary to give the bacterial cells themselves. In diphtheria, for example, immunity can be secured by filtering the broth culture of bacteria, and inoculating the filtrate which contains the diphtheria toxins without any organisms at all, not even dead ones. A very small amount of this diphtheria toxin injected into the arm of a child will give immunity to diphtheria. (See p. 99.)

In all these cases of immunity described, the body reacts much as if the disease had been contracted "naturally." The laboratory part of the process is concerned only with the careful preparation of the material to be injected: to obtain pure culture of the desired organism only, and to make sure that it has been

sufficiently diluted or weakened, thoroughly killed, etc. The resulting protection or immunity following the inoculation of such substances is due entirely to the reaction of the person inoculated, and we therefore speak of the immunity thus gained or acquired as active or "acquired" immunity.

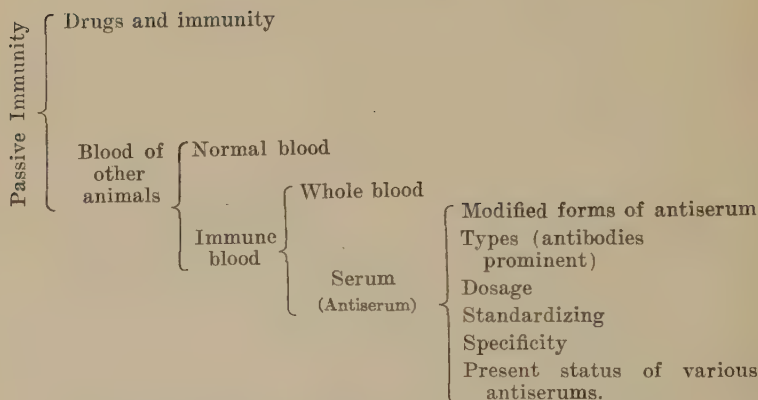
STUDY SUGGESTIONS

1. What are the main types of body reaction to disease organisms?
2. Consult other texts and find a disease where increase in the white corpuscles is commonly associated with that disease.
3. Classify the antistances aiding in the destruction of bacteria.
4. Consult text books of bacteriology (or later chapters in this book) and list the diseases in which the body responds to the infection by making (a) antitoxins; (b) agglutinins or precipitins; (c) opsonins; and (d) lysins.
5. Prepare a list of diseases common in school children (or in adults in industrial concerns, etc.) and tabulate them according to the incubation period. What rules can you formulate to insure the welfare of the people associated in the group you selected?
6. Consult such a source as U. S. Public Health Service Bulletin 436 and list the diseases for which a definite period of immunity is known. Do your state laws for smallpox vaccination, typhoid vaccination, etc., recognize these facts?
7. What factors may affect the virulence of an organism? Illustrate two.
8. Support the old saying "Like cures like" in the treatment, often successfully tried in treating such infections as "chronic boils" due to staphylococcus infections.
9. Plot, as on page 58, the body and bacterial relationship in the last disease of a given member of your family, showing in days on the base line the length of the incubation period, the immune period following the infection, etc.
10. Recently, experimental work with animals demonstrated the reduction or disappearance of various bacteria deposited into isolated or tied off loops of the intestine; for example, typhoid bacteria were greatly reduced in number in five hours; spores of the hay bacillus disappeared completely in twelve hours. Can you cite any other antibacterial mechanism of the body not explainable by the responses described in this chapter?
11. Wright once said: "The physician of the future will be an immunizer." With regard to what disease has that been practically true for a generation? How far is it at present true for typhoid?

CHAPTER III

PASSIVE IMMUNITY

(And Antiserums)



Drug Treatment.—Certain diseases may be prevented or cured by drugs. For example, quinine may be used very successfully in treating human malaria (Figs. 42, 43) as it kills all forms of the malaria organisms, except perhaps the crescent stage (Fig. 44).

When drugs are so used they are usually injected into the blood (intravenously) or into the tissues (subcutaneously) where they are quite promptly absorbed into the circulating blood stream. In certain diseases, however, as in malaria, satisfactory results may be obtained by simply swallowing the drug, quinine being absorbed from the alimentary canal readily enough to secure the desired effect.

Drugs may be used against a variety of types or classes of micro-organisms: iodine compounds against a fungous infection (sporothrichosis, Fig. 45); emetin against protozoa (amebic dysentery) or arsenic-benzol compounds against syphilis, another protozoan disease: chaulmoogra oil against the leprosy bacteria, and iodine, aniline dyes, etc., against various bacteria found in wound infections.

Most of the diseases in which drugs are used effectively are caused by protozoa. Bacteria are apparently much more resistant to most drugs than our own body tissues are. Good results have been claimed in human anthrax through the same arsenic-benzol compound that is used in treating syphilis, and bacteria-infected wounds are undeniably treated advantageously with such drugs as the modified hypochlorites used in the Carrel-Dakin method, or ordinary wound disinfectants. Nevertheless, in the body itself, chemical treatment against bacteria is not yet satisfactorily established. Amounts too small to kill bacteria may affect the body tissues—ultimately if not directly. The treatment of syphilis affords an illustration of such indirect interference in the effect on the teeth following the continued use of mercury.

Recently much time and money have been spent in trying to find drugs which are effective in the animal body against such bacteria as the streptococcus and pneumococcus organisms. For these investigations many new drugs were made—absolutely new chemical substances never before seen, even as laboratory curiosities. The negative results obtained in these exhaustive studies have been most disappointing, indicating that drugs cannot be satisfactorily used in such infections.

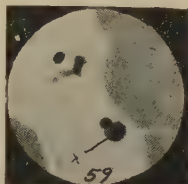


FIG. 42.—Malaria organisms (59) free in blood stream, æstivo-autumnal malaria.

LAWSON *Journal of Experimental Medicine*, Rockefeller Institute.

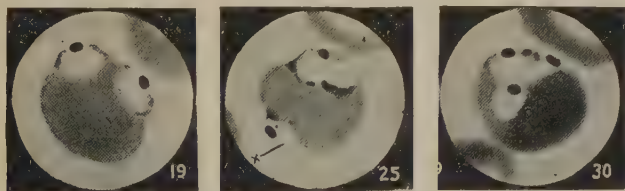


FIG. 43.—Malaria organisms, showing multiple infection of red blood corpuscles. LAWSON, *Journal of Experimental Medicine*, Rockefeller Institute.

Drug Prevention not True Immunity.—While suitable dosage with a drug may prevent or cure a given disease, the subsequent freedom from infection thus obtained is not due to the internal processes or mechanism understood by the term im-

munity. It does not depend upon the presence or increased development of antibodies or white corpuscles.

Immunity by Transfer of Blood Substances.—Since the blood of a person recovering from a disease usually contains a

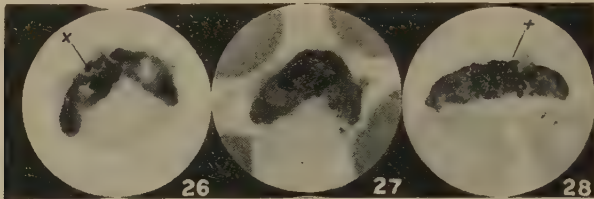


FIG. 44.—Crescent stages of æstivo-autumnal malaria organisms, seen lying in the blood between the red corpuscles.
LAWSON, *Journal of Experimental Medicine*. Rockefeller Institute.

superabundance of special antibodies as described in Chapter II, it is to be expected that immunity thus actively acquired by one individual could be transferred to a second individual by trans-

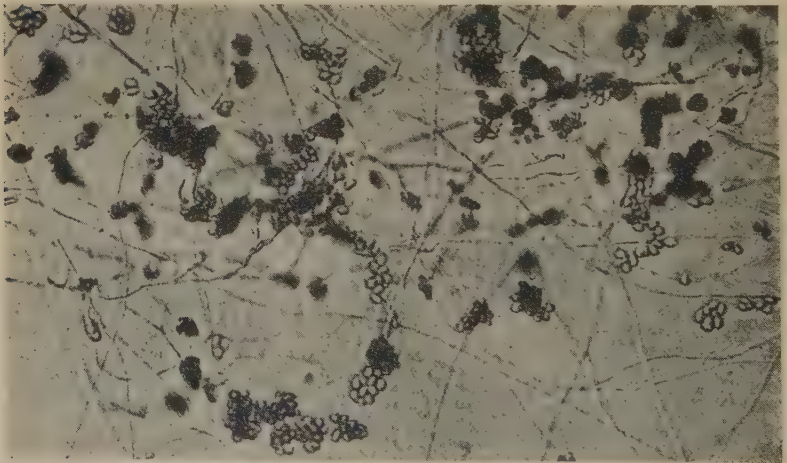


FIG. 45.—A mold, *Sporotrichum*, showing branching mycelium and spore clusters photographed from an old agar plate culture. (WOLBACH, SISSON and MEIER, *Journal of Medical Research*.)

ferring to him a sufficient amount of the blood containing these antibodies (Fig. 46). The second individual thus gains immunity through no activity of his own tissues, and his immunity

is therefore described as passive immunity to distinguish it from the active immunity of the first individual whose blood was so transferred.

Active immunity is always due to the reactions of the individual's own body against organisms (live or dead) or their products (toxins, poisonous split-proteins), while passive immunity is always due to the introduction of blood or serum from another animal which has previously developed an active immunity. In other words, vaccines or actual attacks of disease excite active immunity, while antisera give passive immunity.

To be exact, however, the second individual rarely, if ever, fails to make some active response to the invading organisms himself. The blood transferred, contains sufficient antibodies to counteract or destroy most of the organisms and their poisons, and therefore lessens markedly the active reactions the second individual must make to recover from the disease. It is, however, quite customary to speak as if passive immunity were the only consideration; whereas it really only supplements the injected individual's efforts to

develop active immunity. Indeed, some bacteriologists go so far as to say, "We never by drugs or antisera cure any disease; we can only help the body cure itself."

Immune Substances in Normal Serum.—(See also p. 45.) The serum of a normal individual who has not had a given disease contains so little of any antistances per cubic centimetre that it is, of course, rarely used to combat any specific infection. In the recent infantile paralysis epidemic of 1916 (Fig. 47) normal serum was tried in many instances because blood from recovered or known immune cases could not be procured in sufficient amounts. The blood used was taken from normal adults on

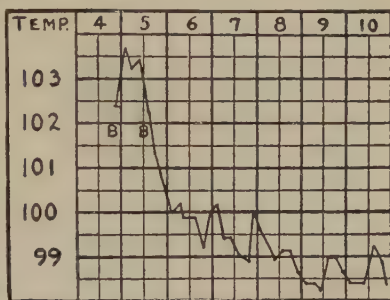


FIG. 46.—Typical temperature curve in case of poliomyelitis, in which immune serum was given, and which ended in complete recovery: B marks the two injections of 15 c. c. immune serum.

ZINGER, Collected Studies, Bureau of Laboratories, Health Department of the City of New York.

the theory that since adults were on the whole much less susceptible and since the adult members of the patient's family had not contracted the disease, the blood of such adults probably contained enough immune substances to benefit the child ill with infantile paralysis (See also Fig. 46).

Since antibodies may accumulate in almost unbelievable amounts in the blood of immune animals (as high as 200,000 times the amount present in normal blood), every effort is made

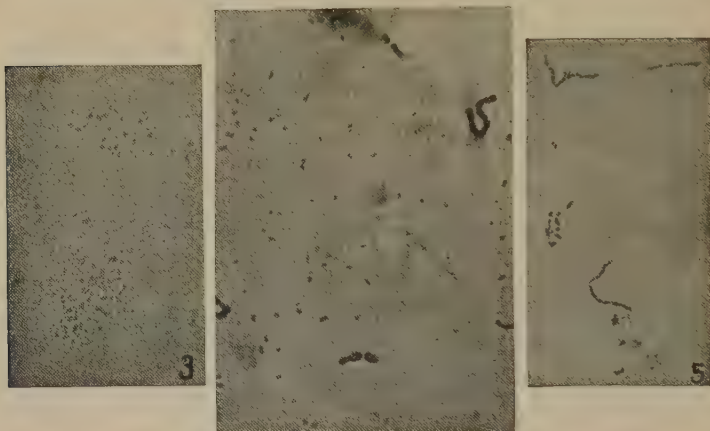


FIG. 47.—Micro-organisms causing infantile paralysis. Separate globoid bodies, 3; paired globoid bodies with long and short chains of *Streptococcus pyogenes* for contrast, 7; chains and pairs of globoid bodies, 5. (x 1000) FLEXNER AND NOGUCHI, *Journal of Experimental Medicine*, Rockefeller Institute.

to secure blood known to be immune—and it is only in emergencies that normal blood * would be tried at all.

Difficulties Attending Transfer of Whole Blood.—When whole blood is transferred to another individual clots may be formed, clogging the capillaries or exerting pressure upon delicate nerve areas. When the blood so transferred belongs to another animal species, other undesirable results may also occur: (1) the transferred blood cells may be treated as foreign material and digested or destroyed; or, (2) some of the blood proteins—

* This discussion, of course, does not refer to ordinary transfusion of normal blood where increasing the antibodies is not the chief consideration, but where the aim is to replace the loss of large amounts of blood, injured red cells, etc.

even in the liquid serum itself—may be digested as foreign proteins, liberating poisonous substances (See p. 193). All of these possibilities—or rather probabilities—make it desirable to lessen the amount of foreign substances injected; therefore, when blood is to be transferred to give immunity not only are the corpuscles first removed from such immune blood, but the fibrin also is removed from the plasma, leaving the serum only to be used for injection (Fig. 49). This immune serum, since it contains the desired antibodies is called *antiserum*. Quite commonly, however, the shorter and less accurate term *serum* is used for an immune serum. Serums are, as might be expected, much less irritating than whole blood; a discussion of serum sickness is given in the chapter on anaphylaxis, but at present it is sufficient to say that the fever, rashes, etc., are much less common and less severe than formerly when whole blood was used for injection.

Modified Serums.—It is an advantage to remove not only the corpuscles and fibrin as described above, but as much as possible of the protein substances still unavoidably left in the serum. This is done in diphtheria antiserum by precipitating out some of the proteins with special chemicals such as salts. (See p. 94.) While some of the antibodies are lost in such modification of the serum, the remaining antibodies are concentrated to such a degree that the resulting fluid contains four to six times as much in a given bulk, and any irritation following the injection of the necessary dosage is much less than when the greater volume of whole serum is used. Diphtheria antiserum is practically the only serum greatly modified before it is injected; other serums such as pneumonia serum, are merely filtered to remove small particles, such as broken corpuscles or shreds of fibrin. (See p. 93.)

Antibody-Extracts.—Recently a complicated and ingenious technic perfected by Huntoon has made it possible to obtain



FIG. 48.—Film from central nervous tissue of monkey inoculated with human virus: this shows pairs of globoid bodies similar to those in the preceding figure. FLEXNER and NOGUCHI *Journal of Experimental Medicine*, Rockefeller Institute.

antibodies practically free from the usual accompanying proteins. This process may be very roughly described by saying that a given antiserum is mixed with its related bacteria (for example, pneumococcus antiserum with pneumococcus organisms) to allow

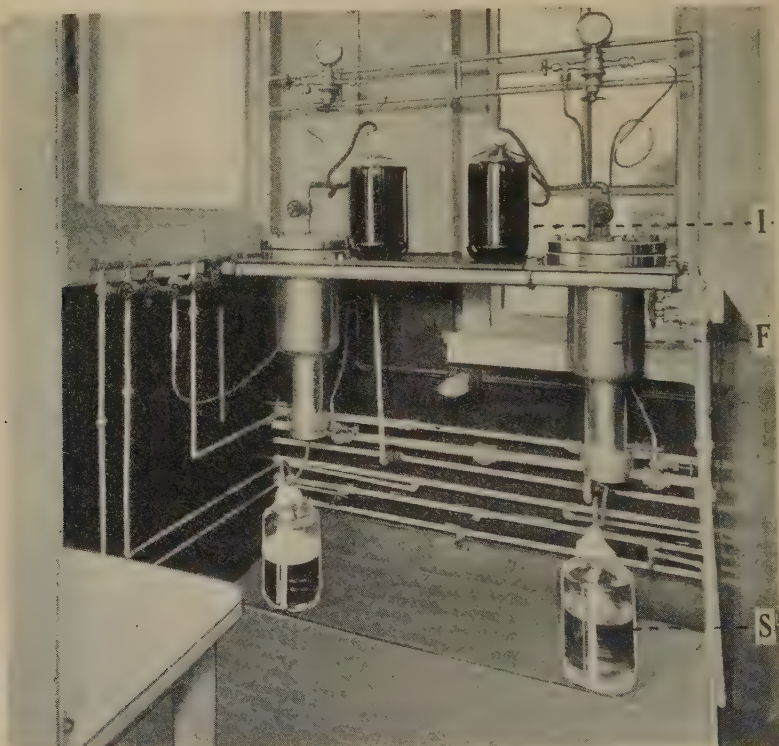


FIG. 49.—Filtering serum to remove broken cells, shreds of fibrin etc. before injection; *I*, immune serum; *F*, a porcelain filter (see following illustrations); and *S*, the filtered serum, ready to bottle for use. H. K. Mulford Co.

them to absorb out the allied antibodies. Then this bacteria-antibody combination is treated to disassociate the bacteria from the antibodies. This disassociation and removal may be accomplished by proper treatment with salt solutions, dextrose solutions, or even distilled water. On centrifuging, a clear or water-white solution or antibody-extract is obtained which is almost

free from serum proteins and therefore strikingly free from the usual more or less irritating effects connected with antisera. (See p. 71.)

Some of the antibodies are "lost" in this method of treatment (all are not disassociated from the bacteria, etc.). But the concentrated antibody-extract thus obtained is very readily available in the body; and surprisingly small amounts (50 c.c. in human cases) have given very promising results; for example, a prompt initial drop in temperature (from 104° F. or 107° F. to 98° F. in 8 to 12 hours), and a decided shortening of the period of illness—both in the pre-crisis interval and in the total period of illness. Encouraging results of this kind were recently reported in fifty hospital pneumonia cases, and extensive trials of pneumococcus antibody-extracts are now being made in various hospitals in the eastern United States.

Terminology Relating to Antisera Misleading.—Such a modified or concentrated serum as diphtheria antiserum is primarily an antitoxin, and so is usually called diphtheria antitoxin rather than diphtheria antiserum. In such antisera as tetanus antiserum, or gas gangrene antiserum, no such modification or reduction in bulk takes place, and whole serum is used. Each of these, too, however, is called

antitoxin simply because its main action is antitoxic, acting against the tetanus or the gas bacillus toxin.

Then, too, the first antisera of practical use were mainly antitoxic in their action, and it was therefore natural to use the

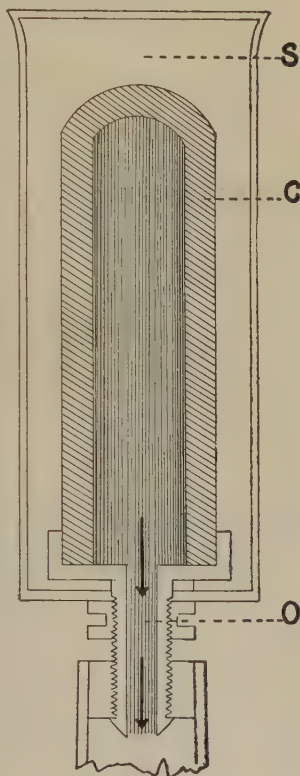


FIG. 50.—Section through a porcelain or clay filter showing the outer metal case, the space into which the substance is poured (S) the porous clay or porcelain filter (C), and the outlet for the substance which has passed through the porous filter (O). See following illustration.

two terms interchangeably. All this often leads to a confusion regarding the terms antitoxin and antiserum. Antiserum is the larger term; some antisera contain antitoxins, and some do not, but owe their value to antibodies other than the antitoxins, such as the opsonins.

Types of Antisera.—The blood of immune animals contains more than one reacting substance or antibody, each of which is discussed separately in the following chapters. In immune blood or sera, as already described (p. 49), one anti-

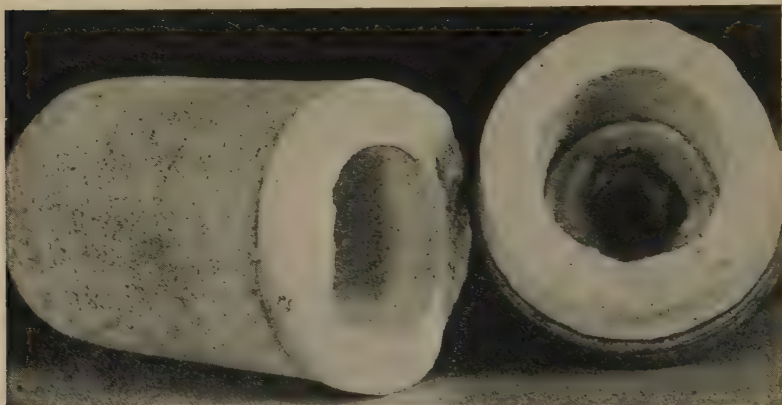


FIG. 51.—A porcelain filter "candle" broken across to show relative thickness of the filtering "candle", its porous character and the opening through which the filtered liquid passes into the vessel beneath. HEINS.

body is usually much more important than the others; sometimes so much so that little attention is paid to any of the other anti-substances as in the case of the antitoxin in tetanus antiserum.

Antitoxins, as stated in a previous paragraph, are the most prominent antibodies in the immune sera used in treating tetanus, diphtheria and gas gangrene infections.

Opsonins, which aid the white corpuscles in their work, are very important in most of the other antisera now in use. Meningitis, pneumonia and streptococcus antisera have remarkable stimulating effects upon the action of white corpuscles, though meningitis antiserum is partly bactericidal (lysins) and pneumonia antiserum has also definite bactericidal and antitoxic values. Recently, very different substances, anti-endotoxins

or anti-split proteins, have been advanced as "exceedingly important in pneumonia, meningitis and streptococcus immune serums."

Despite the high amount of agglutinins developed in certain diseases, such as typhoid and dysentery (Fig. 52), there are as yet no established antisera where the passive immunity obtained is claimed to be mainly due to agglutinins.

The efforts to secure antisera for use in protozoan diseases have been much less successful than with diseases of bacterial origin. The most effective so far is probably antirabic serum, where a slight inhibiting power is granted. In scarlet fever and measles, for both of which protozoan origin has been claimed though not proven, beneficial results are reported from serum treatment, but such treatment has not advanced beyond an experimental stage in these diseases. In most of the human protozoan diseases, including syphilis, no benefit has yet been demonstrated from the use of antisera.

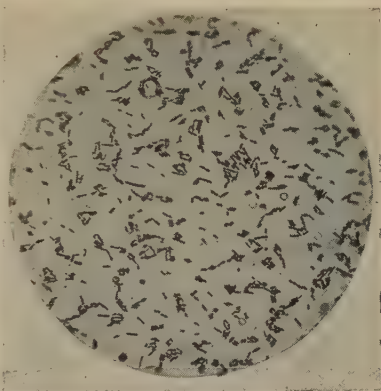


FIG. 52.—Shiga's dysentery, broth culture, (x 1500). (SANDS.)

Other Antisera.—Fairly successful antisera have been obtained against other substances not of bacterial origin, such as cobra and rattlesnake poisons. Flower pollens have been less successfully used in producing an antiserum for curing or preventing hay fever.

Dosage.—The dosage varies considerably with the aim in view, a much smaller dose being given to protect against possible infection than to cure disease when already established. For example, nurses and others in contact cases of plague are given immunizing doses of 20 c.c., while the patients themselves may be given five times that amount, 100 c.c.

The amount of anti-serum necessary to give protection depends upon such different factors as (1) the stage and virulence

of the infection, (2) the concentration of the antibodies in the serum, and (3) the method by which the antiserum is injected (directly into a vein, into subcutaneous tissue, etc.).

The importance of the first factor may be illustrated by such a statement as the following: Four times as much rattlesnake antiserum is necessary for protection one hour after the person has been bitten as if administered at the time of the bite. Or, the initial dose of diphtheria antitoxin varies from 5,000 to 10,000 units (See p. 97), and in pneumonia from 60 to 100 c.c., depending upon the condition of the patient and the stage of the infection.

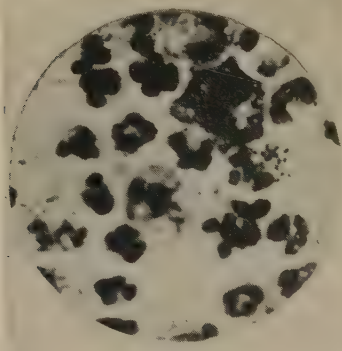


FIG. 53.—Spinal fluid showing meningitis organisms and white corpuscles. Bull.

6 times the original number of units. It would, therefore, be quite possible with some antitoxin to give an ordinary dose, 5,000 units, in less than 1 c.c. of modified serum.

The third factor, method of injection, modifies very greatly the amount necessary for protection. In tetanus, for example, the New York Research Laboratory recommends 5,000 units intraspinally or 10,000 units intravenously. This difference in amount is due mainly to the relative rapidity with which the immune substances can reach the critical areas, in this case the central nervous system. If given intramuscularly or subcutaneously still larger doses would be necessary, 100,000 units or more. There would, of course, be some doubt as to whether even such an increase in the amount injected could adequately compensate for the slower absorption rate.

The second factor, concentration of the antibodies in the serum, varies with the antibody strength of the original serum itself, and with the degree of later modification of the serum. The serum as obtained from the horse varies from 150 to 1,000 units per c.c., although but a small fraction of the horses yield as much as 800 units per c.c. When modified as described for diphtheria antiserum, the decreased bulk means a strength of from 4 to

Standardizing Antiserums.—The value or strength of an antiserum depends, of course, upon the antibodies it contains. Although the action of these in the body may not be proportional to the measurement standards that we can apply in the laboratory, we are nevertheless often dependent upon such laboratory procedures.

Measuring Protective Values Directly.—Where serums can be definitely standardized the strength is usually estimated by determining how much is necessary to destroy or neutralize the known fatal dose of the related toxin or bacteria. In diphtheria, for example, the amount to protect a medium-sized (250 gm.) guinea pig against the known fatal dose of diphtheria toxin is determined by inoculating each of a series of guinea pigs with varying combinations of the antiserum to be tested and the known fatal dose of diphtheria toxin. This protective amount is very small—but a small fraction of a cubic centimetre—and not nearly enough to protect a human being; therefore 100 times the minute amount that protects a guinea pig is used as the standard diphtheria antitoxin unit. This insures a workable unit—one that can be handled with greater facility and accuracy.

In tetanus the unit is 1,000 times the amount needed to protect a larger (350 gm.) guinea pig against the known fatal or lethal dose of tetanus toxin. The strength of pneumococcus antiserum is determined by finding its protective value against pneumococcus organisms when both are injected into mice.

Measuring Specific Antibody Content.—Besides determin-

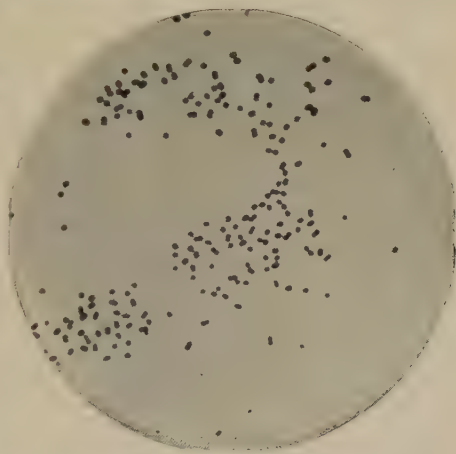


FIG. 54.—*Neisseria catarrhalis* (*Micrococcus catarrhalis*) from a nasal suppuration, one of the organisms sometimes causing meningitis ($\times 1200$).
LEWIS, *Journal of Pathology and Bacteriology*.

ing the *protective* values, some idea of the strength of a given antiserum may be obtained by testing it for its relative abundance of the antibodies known to be prominent in that particular kind of antiserum—by determining, for example, the agglutinin content of dysentery antiserum.

In this method, the laboratory processes are not so simple nor, apparently, so parallel to what takes place in the body. Nevertheless, the determination of the relative abundance of opsonins, agglutinins, etc., present in a given antiserum constitutes the only definite standard in sight, and, therefore, we

endeavor to determine in different antisera the relative amounts of the most prominent antibodies present. Such laboratory measurements are usually made for one type of antibody only, such as opsonins; and, obviously, the results obtained tell us nothing of the possible aid rendered by the other antibodies, such as agglutinins, or lysins.

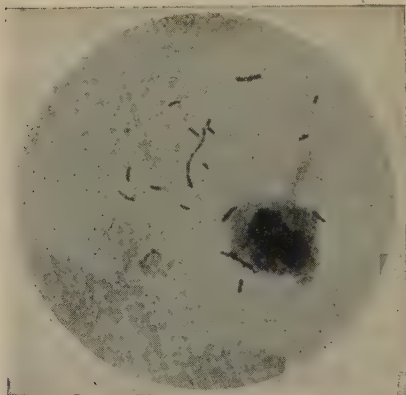


FIG. 55.—Smear of spinal fluid sediment showing pleomorphic forms of the influenza bacillus ($\times 1200$) from a case of meningitis. ART AND TUMPEER, *American Journal of Diseases of Children*.

Other Methods of Estimating Values.—The strength of some antisera can not be accurately determined by either of these

two methods, and we are compelled to use such admittedly imperfect standards as their transparency when diluted in certain proportions.

Since most antisera have not yet been satisfactorily standardized, the treatment varies with the patient's reaction or condition after a trial dose of a few cubic centimetres has been given. This is, of course, very unsatisfactory, as the actual bulk means very little without some way of determining the antibodies present in a given volume. Probably the dosage is usually too small, and the promising effects recently obtained in treating pneumonia are attributed to the greatly increased amounts of antiserum given, sometimes over 100 c.c. at a time.

Number of Doses.—While a single dose is usually sufficient,

as in diphtheria, we are not yet able to measure the strength of other antiserums definitely enough (See standardizing antiserums following) to calculate the required dosage so exactly; then, too, we have not yet accumulated such a wealth of experience with them. The practice, therefore, in such cases, is to give one large dose (10-25 c.c. for meningitis, 100 c.c. for pneumonia) and base the later dosage upon the patient's subsequent condition. Doses may be repeated every 10 or 12 hours until improvement is evident, or they may be repeated daily for as long as 4 to 6 days. Occasionally, the treatment may continue for as long as two weeks.

In the *preventive* use of antiserums, the repetition intervals are based on the rate at which the injected serum substances are eliminated from the body. Nurses in contact with diphtheria cases may be given diphtheria antitoxin every 8 to 10 days, and wounds likely to be infected with tetanus organisms may be so treated at similar intervals.

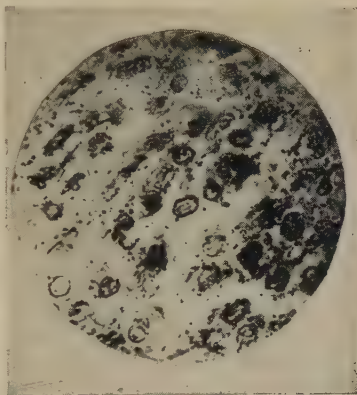


FIG. 56.—Spinal fluid showing heavy invasion of spinal fluid in meningitis. Bull.

Variations in Disease

Organisms.—Difficulties often arise because organisms enough alike to be given the same name and enough alike to cause the same general clinical symptoms, are not, nevertheless, exactly alike in all their effects on the body. There are, for example, several types or varieties of the pneumococcus organism (*Diplococcus pneumoniae*) responsible for pneumonia, and, similarly, several varieties of paratyphoid organisms, *Bacterium paratyphosum*, which cause intestinal disturbances. Even more varieties are probably represented in streptococcus infections. It is thought that such variations in pneumococcus organisms may be responsible for second attacks of pneumonia. An individual recovering from one variety of pneumococcus has made anti-substances against that type only, and is not fully protected against a second or different type. Nor would his blood confer

complete protection upon another having an attack of pneumonia due to different types of pneumococcus.

In producing antisera, therefore, some attention must be paid to the varieties of the organism responsible for the disease or infection. If it does not mean too great a delay, the type of such organisms as pneumococcus and dysentery is first determined by such procedures as obtaining from the patient pure cultures of the organism and finding its reactions in various media and against various blood serums. (See also p. 122.) This may make

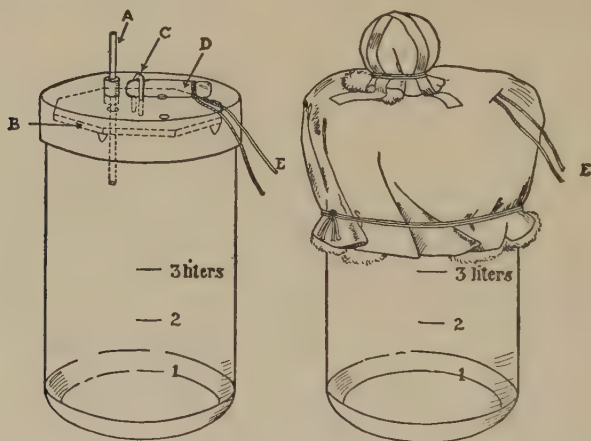


FIG. 57.—Bleeding jars for collecting serum. *A*, tube for receiving blood; *C* and *D*, device for allowing fall of weight, *B*, and causing the depression of the clot. Serum is then withdrawn through *A*. This method yields about 50% serum. AVERY, CHICKERING, COLE and DOCHEZ, Monograph, Rockefeller Institute of Medical Research.

it possible to select an antiserum made in response to the patient's own type. Such antisera are by far the most satisfactory. Even with pneumococcus, however, where at present the most definite "typing" has been done, effective antiserum has been produced for but two of the four recognized types of pneumococcus organisms.

Since in most cases such "typed" antisera are not available, a choice must be made between two other procedures. First we may ignore variety or type differences and select the most virulent or the most common type of an organism with which to inoculate an animal for the production of antiserum. The use of such

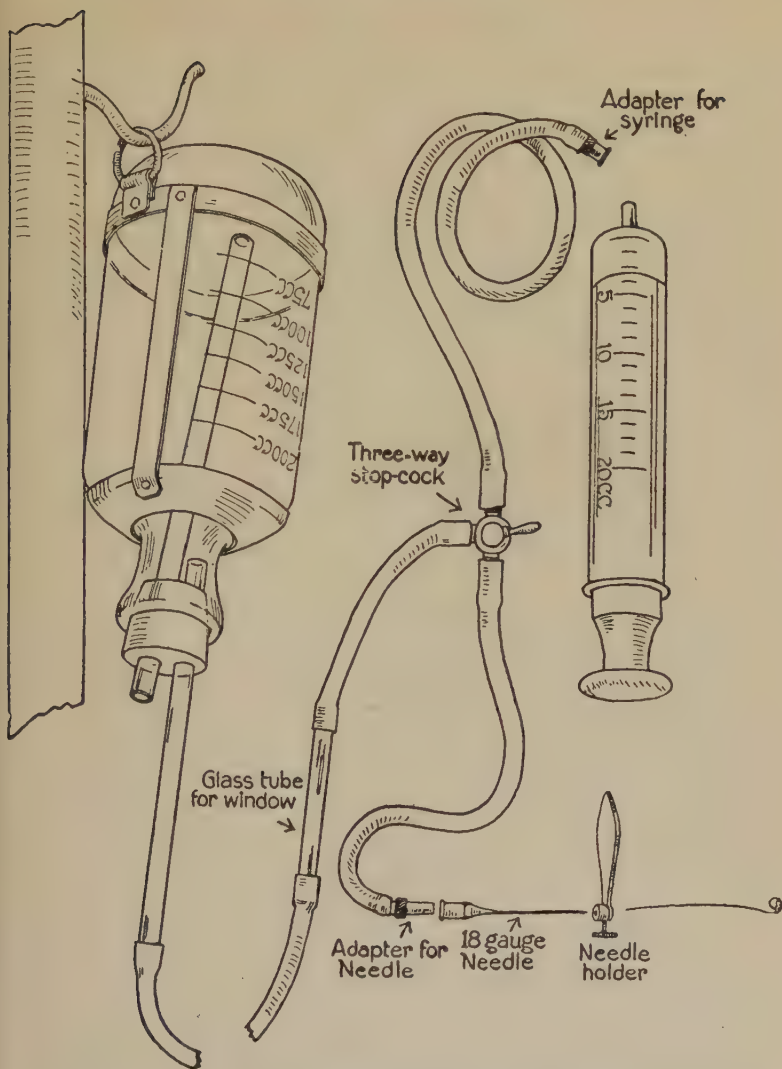


FIG. 58.—Apparatus for injecting serum, showing the bottle of serum, suspended from the bed or wall. The serum may be drawn into the syringe and so injected through the needle into the vein, or the syringe may be left out of the scheme and the serum allowed to flow from the bottle into the vein by gravity. The serum can be kept at a body temperature by placing the tubing between two hot water bags. AVERY, CHICKERING, COLE AND DOCHEZ. *Journal of Experimental Medicine*, Rockefeller Institute.

an antiserum for an infection due to another type of the same organism is based upon the hope that because of the general likeness between the types the antiserum thus obtained may contain antibodies so closely approaching the kinds desired that they will aid in overcoming the infection. By the other method of procedure we may prepare a polyvalent serum as described in the next paragraph.

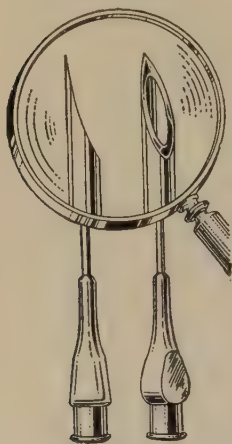


FIG. 59.—Tip of syringe needle, magnified to show that it is a hollow tube, with a bevelled point. This general type of point is used both for drawing blood or serum, and for injecting vaccines and serums. H. K. Mulford Co.

Polyvalent Serums.—To obtain a polyvalent serum an animal is inoculated with several varieties of the same organism. An animal may thus be made immune to several different varieties or types of a given organism such as the dysentery bacteria. This means that its blood contains antibodies against all the varieties or types with which it was inoculated, and is of value in infections due to any one of the several varieties. Such an antiserum is called a polyvalent serum; literally it means a serum of *many powers* or *much strength* or a serum of many combining powers. Polyvalent serums are used more in dysentery, meningitis and gonococcus infections than in other diseased conditions. While such polyvalent serums have a wider range, there is of course an accompanying disadvantage in that this very range of contained antibodies may mean an increase in the unnecessary foreign proteins injected. Then, too, there is always the possibility that such “polyvalent” antisera are not truly polyvalent—that the animal producing the antiserum did not react to all of the varieties with which it was injected.

Mixed Antisera.—Antibodies can even be produced simultaneously in the same animal against a group of quite distinct organisms, such as pneumococcus, streptococcus and influenza organisms, or against typhoid, dysentery and the “colon” bacteria. Such antisera would—because of their wider range—have a wider margin of possibly unnecessary substances and they are, therefore, open to the same objections given above for polyvalent serums. While a few mixed anti-

serums are on the market, there is none of undisputed value, and the production of mixed antiserums must be considered as still in the experimental stage.

Present Status of Antiserum Treatment.—It is clear from the preceding discussion that the value of antiserum treatment depends upon three things: (1) the accuracy with which the causal organism is determined, enabling thereby the selection of the corresponding antiserum; (2) the promptness with which such determination can be made, for to be effective an antiserum must be administered before the tissues are too greatly injured, and (3) whether a potent antiserum has yet been prepared.

In both meningitis and pneumonia the situation is complicated by the fact that the causal organism may be of a wholly different type (Figs. 53 to 56) from the one usually present in such infections: *Neisseria meningitidis* (*Diplococcus* or *Micrococcus meningitidis*) in meningitis and *Diplococcus* in pneumonia. It is hardly necessary to state that antistances formed by the horse against the coccus organisms ordinarily causing meningitis cannot be expected to counteract the conditions found in meningitis due to tuberculosis organisms; similarly, a serum successfully used in combating pneumonia due to one of the types of pneumo-

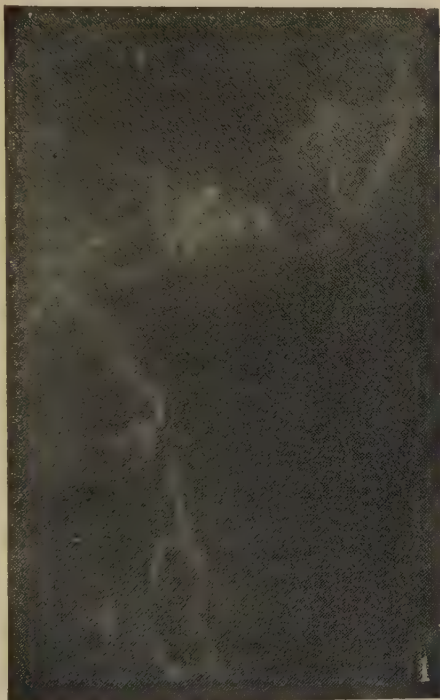


FIG. 60.—Dark field photograph of a "filterable virus", *Leptospira icteroides*, the causal organism of yellow fever, from a two-week culture on semi-solid rabbit serum agar, (magnified 3000 x). NOGUCHI, *Journal of Experimental Medicine*, Rockefeller Institute.

coccus, *Diplococcus pneumoniae*, will be less or not at all helpful when the pneumonia is due to streptococcus bacteria. It is to meet such difficulties that mixed antisera are tried.

At present there are but four infections in which antiserum treatment is on a practical and unchallenged basis. For two of these diseases the antisera are antitoxic—diphtheria and tetanus. For the other two diseases the sera are antibacterial—the antisera used in treating meningitis and (two types of) pneumonia.

Although the results obtained are less uniformly successful, antisera are prepared and used for several other infections. Dysentery, especially the Shiga type of dysentery (Fig. 52), often responds very favorably to antiserum treatment. Good results are reported with anthrax and plague, both fortunately uncommon in our country.

Benefit has been obtained from appropriate antisera against uncomplicated cases of “gas gangrene” or wound infections so prominent in the recent war. In certain types of streptococcus infections some aid may be gained by the use of antisera.

Whole blood from scarlet fever convalescents has been used in scarlet fever with apparent advantage, although as before stated, this treatment is still in the experimental class. Neither blood nor antiserum seems to give definite aid in treating infantile paralysis. (See p. 69.)

More detail concerning antisera—their contained antibodies and the diseases in which they are most helpful—is given in the several chapters following. In each case the discussion includes also a brief mention of the days in which the antibodies may be used as aids in diagnosing disease.

STUDY SUGGESTIONS

1. List the diseases, with the causal organisms in each, in which drugs afford helpful treatment. How many of them are of protozoan and how many of bacterial origin?
2. What is passive immunity? Give an example of active and also passive immunity against diphtheria or against meningitis.
3. What antibodies may be important in the antisera given to afford the patient passive immunity?
4. Show why the whole blood of an immune animal may be more irritating when transferred to a patient than the serum of that immune animal.

5. Show that antiserum is a broader or more inclusive term than antitoxin.
6. Describe one way of standardizing or determining the strength of an antiserum.
7. List the antisera in a commercial catalog of such biological products; how many of them are used in the hospital or by the physician you know best? Can you cite one instance probably indicating too conservative an attitude on the part of the medical authorities? Cite one, indicating the sale of a preparation not yet certainly past the experimental stage.
8. "Disinfection, isolation, and the widening of the danger space between the sick or infected and the well is the chief occupation of modern sanitation," according to Dr. Theobald Smith. After reading Chapters II and III name the practices of preventive medicine which widen the danger space.
9. Difficulty in finding animals sufficiently susceptible to human disease organisms has limited our ability to prepare antisera for human protection. To what human diseases is the horse sufficiently susceptible to aid in this way?
10. An early report (October, 1921) by Noguchi contrasts the usual mortality of yellow fever, 50 to 60 per cent. with that obtained in 152 cases treated with an antiserum, with a mortality of but 9 per cent. How does that compare with the helpful results obtained with diphtheria antitoxin?
11. In a record of 1200 cases of meningitis the death rate varied with the promptness with which treatment was begun as follows: 1-3 days, 18 per cent. died; 4-7 days, 27 per cent. died; and after 7 days, 36 per cent. Since among the untreated, the deaths are 80 per cent., work out for each group the treated child's chance of recovery when compared with the untreated child.

CHAPTER IV

TOXINS AND ANTITOXINS

Toxins and Antitoxins	Toxins	Definition	Bacterial toxins	Double toxins
				Modified toxins or toxoids
			Production requirements	
			Excite antitoxins against	Poisonous substances { Animal Plant Non-poisonous substances
	Uses		Produce antitoxins for man in other animals	Production of antibodies Modification of antiserums Standardization Dosage
		Produce antitoxin in human beings	Antitoxin modification (Toxin-antitoxin) Treatment of infants Efficiency of toxin-antitoxin treatment.	
		Test of susceptibility (Schick test)	Typical and pseudo-reactions Value of the Schick test	
Present status of antitoxins			Diphtheria	
			Tetanus	
			Other diseases	

Toxins.—Bacterial toxins have already been described briefly in the first chapter of this text. Toxins of such organisms as tetanus and diphtheria are obtained by filtering a broth culture of the respective organisms. The filtrate contains the toxins; it is spoken of as toxin, as if it were toxin only, although its chief bulk consists of water and soluble food substances.

This filtrate may contain more than one kind of toxin. For example, two distinct toxins are quite constantly formed by the tetanus bacillus (Fig. 61): one, affecting mainly the motor nerves and associated with the characteristic lockjaw or tetanus convulsions; and a second, causing a dissolution of the red blood cells. Similarly the filtrate of diphtheria cultures contains not only the specific diphtheria toxin which is the cause of the usual acute symptoms of diphtheria, but also a second toxic substance or toxon, slower in its action and responsible for the paralysis which sometimes occurs in the late stages of diphtheria.

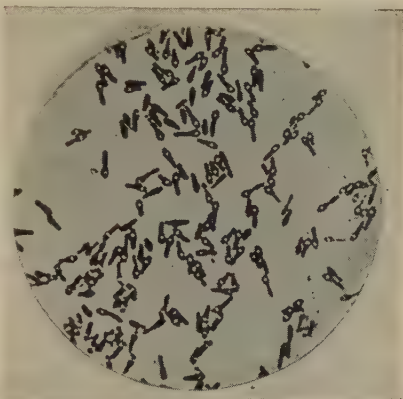


FIG. 61.—Lockjaw or tetanus organisms most of which have formed spores, the lighter bodies at the swollen end of the cell.
Willams, Brown and Earle.

In an elementary text such as this, little emphasis can be placed upon these separate toxins, and the term toxin is used in a general inclusive sense. The same is true of the related term antitoxin; no recognition is here given to the fact that the protective antitoxic action of the blood in a recovered diphtheria case, for example, is due to at least two antitoxins, each acting against its respective diphtheria toxin.

Toxon.—A secondary or lesser toxin is sometimes termed toxon, to distinguish it from the better known, stronger or more typical toxin. More than one such toxon may be produced by the bacteria during their development, whether growing in the body or in ordinary culture media.

Toxoids.—Another similar-sounding term sure to occur in all

reference books on immunity is the word toxoid. Toxoid is quite consistently used for toxins that have changed with age, temperature conditions, etc., thereby becoming less poisonous, but not losing their power of chemical combination with the body cells or with antitoxins (Fig. 62). Such changes may be considerable; toxin but a few months old may lose more than half its strength by conversion to toxoids.

Toxoids are mentioned here because their presence may affect the amount or bulk of a given antitoxin which it is necessary to give for protection, as in the toxin-antitoxin treatment described on page 98. Apparently, when antitoxin is injected into the body it first combines with the toxoid present, then with the toxin and last with the toxon. If a large amount of changed

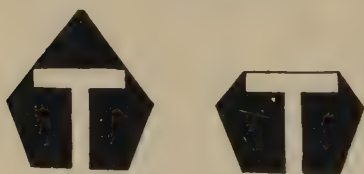


FIG. 62.—Left, a toxin particle; and right, a toxoid or changed toxin which has lost part of its toxic quality but still retains its ability to combine with tissue cells.

toxins or toxoids is present, such unsuspected toxoids may thus combine with the antitoxin, leaving too much free active toxin. A similar difficulty may be met in attempting to arouse active immunity; the individual receiving a toxin-antitoxin mixture contain-

ing such toxoids might not be sufficiently protected against the contained toxins even though the mixture contains only the usual amount of toxins.

Non-bacterial Toxins.—Since bacterial toxins have never been satisfactorily analyzed and no chemical formulas can be given for them, it is natural that the term toxin should be given to substances from very different sources if they are like bacterial toxins in the two most striking characters: (1) in having very irritating or toxic effects and (2) in exciting the production of antibodies.

From this point of view we may have several different types of toxins. Some of these are commonly recognized as poisons, such as the poisons formed by certain species of snakes, fish, eels, spiders, etc. Another type is illustrated by the characteristic substances found in certain plants, such as the poisonous ricin in the castor oil seed, or abrin in the Indian licorice bean. As a third illustration of the substances against which antitoxins may

be formed we have substances not ordinarily toxic or poisonous, such as the normal body enzymes (pepsin, trypsin, steapsin, and lactase).

Our interest, however, attaches mainly to the toxins formed by common human disease organisms, such as tetanus and diphtheria. Therefore, unless otherwise specified, antitoxin as used hereafter refers to the antibodies formed against such bacterial toxins.

Usually the diseases due wholly or in great part to bacterial toxins occur only when the appropriate bacteria develop in the body: *e.g.*, in the intestine in Shiga's type of dysentery; in the throat membranes in diphtheria; and in wound areas in gas gangrene infections. A notable exception to this is found in botulism (Fig. 63); in this disease, most if not all of the cases recently studied have been due to the eating of foods which had already undergone considerable decomposition and in which the accumulated toxin had not been destroyed by the final heating process. (See glossary.)

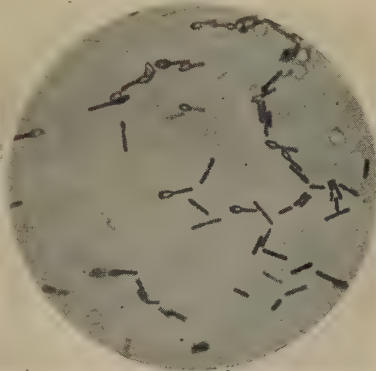


FIG. 63.—*Clostridium botulinum*, the organism responsible for botulism, a type of food poisoning. Reddish.

Antitoxins.—Our main interest in toxins, however, lies not in such modifications or differences but in their common possession of the power to stimulate the production of antibodies. As we have already seen we make practical use of this power on a large scale by injecting appropriate toxins, such as diphtheria toxin, into other animals, so that the blood of such infected animals may later be drawn off to aid human beings (See p. 90). All of the available antitoxin in the blood of such immunized animals is contained, not in the blood corpuscles but in the serum. Using the serum only, therefore, as in the preceding chapter, eliminates the corpuscles and fibrin and so decreases materially the amount of foreign protein substances that must be introduced when an animal's blood is transferred to give passive immunity.

Occasionally this elimination of unnecessary protein substances can be carried still further and the serum itself can be modified.

Modified Antiserum or Antitoxin.—As described in the preceding chapter the only antiserum in which this treatment or modification of the serum does not occasion too great loss of protective substances is diphtheria antiserum. All the other so-called antitoxins (tetanus, gas gangrene and Shiga's dysentery) are really antisera, but as before explained, such antisera are often called antitoxins, because their main action is antitoxic.

Preparation of Diphtheria Antitoxin in Horses.—The animal phases of antiserum production were almost ignored in

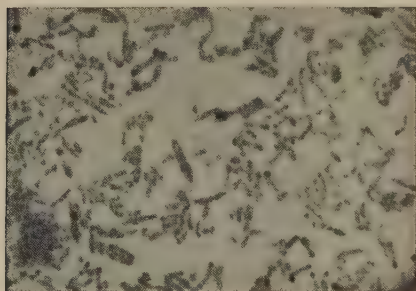


FIG. 64.—Rod-like diphtheria organisms, magnified more than 1000 times, showing distinctly the characteristic unequal staining. EMERSON, Clinical Diagnosis, J. B. Lippincott Co.

the preliminary discussion of the production of antiserum in Chapter III. It may be of interest here, therefore, to begin with the horse and give a little more in detail the whole process of producing antitoxin. For this discussion we have selected diphtheria antitoxin, partly because it is historically the most interesting, as diphtheria

antitoxin was the first antitoxin to be placed upon an unchallenged basis. Diphtheria antitoxin is also more popularly and widely known than any other. The process of production, as described, is, only in a general way, true for other antisera, as there is, of course, considerable variation in such details as the following:

(1) Method of inoculating the animal which is to produce the antiserum—*e.g.*, with dead bacteria or only their toxins; (2) the dosage: amount of material inoculated at a time and the number of inoculations; (3) the time period that elapses before sufficient antibodies are formed to make the use of the animal's serum really helpful.

The preparation of diphtheria antitoxin may be discussed under three headings: (1) The animal phase, beginning with the

inoculation of the animal and extending to the withdrawal of blood containing the helpful antisubstances; (2) the separation of the serum from the other elements of the blood; and (3) any further modification of the serum itself.

(1) **The Animal Phase.**—The horse selected for the production of antitoxin is injected at short intervals, (every two days) with diphtheria toxin. This toxin is nowadays partly neutralized with some diphtheria antitoxin previously made by another horse. As explained on page 99, this makes it possible to give a large dosage, and so secure a greater or stronger reaction by the horse—more antitoxin in each cubic centimetre of his blood. Beginning with a dose of about 3,000 units of toxin, or 10 minimum lethal doses for the guinea pig, the dose is gradually increased until on the last injection the animal may be given about 500,000 units. (It is impossible to describe the dosage or treatment accurately in ordinary terms of

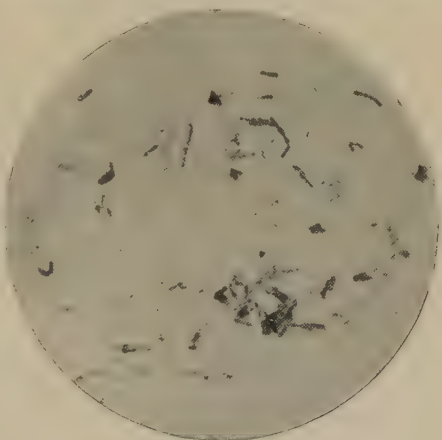


FIG. 65.—A throat smear from a case of diphtheria, fifth day, showing much the same range in the diphtheria organisms, *Corynebacterium diphtheriae*, as the pure culture in the preceding illustration. CAVE, *Journal of Pathology and Bacteriology*.

bulk, because the strength of the toxin varies greatly; those who need some such term of measurement to help them to visualize the process, may picture the initial injection as probably ranging from 10 to 15 c.c.). By the sixth to eighth week the horse is usually producing enough antitoxin to make it worth while to withdraw blood for use in the protection of human beings.

The blood is drawn from one of the prominent surface veins in the neck of the horse, in which the blood is flowing downward on its way back to the heart. Aseptic methods are used both to keep the drawn blood sterile and to prevent the horse from becoming

infected. The skin is cleansed (usually shaven and washed with a disinfectant), and a slender, sharp-pointed metal tube is thrust upward into this vein, the metal tube being connected by means of a sterile rubber tube with a large sterile bottle held below. Eight to ten quarts are often taken at a time. When the bleeding tube is withdrawn the skin is pinched together by the fingers to slow the flow and allow a clot of blood to form at the small puncture made by the tube.

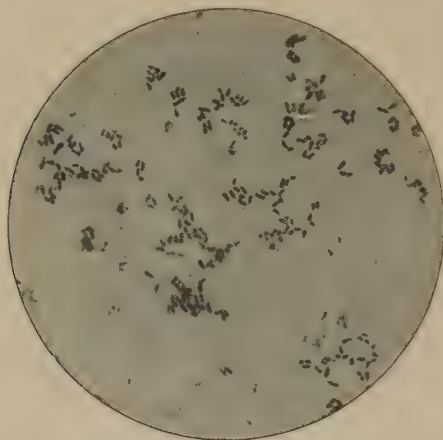


FIG. 66.—The presence of diphtheria-like bacteria, such as the shorter, plumper and more uniform "Hofmann's bacillus" often lead to incorrect diagnosis or interpretation. The above smear made on the twelfth day shows the so-called pseudo-diphtheria only, while the earlier smears, one of which is pictured in the preceding figure, showed true diphtheria organisms. CAVE, *Journal of Pathology and Bacteriology*.

The surface is again washed with carbolic or a similar disinfectant, and the horse, weakened but usually without other ill effects, is allowed to "rest" for several weeks before another bleeding is made. Horses may be bled in this way throughout a period of two years or even longer. Additional doses of diphtheria toxin are given the horse from time to time to increase the rate of production of antitoxin or to

lengthen his productive period. During most of this period the horse seems quite normal, giving little or no evidence of discomfort, and may be used for light work at the station or given other suitable forms of exercise. In one city laboratory a diphtheria antitoxin horse has been used in emergencies for ambulance work.

(2) **Separation of Serum.**—The immune blood drawn into sterile bottles is next treated to separate the white and red corpuscles as well as the fibrin from the resulting liquid or serum. One of the simplest ways of doing this is to allow the blood to stand for twelve to twenty-four hours, in which time it will clot, the corpuscles and fibrin forming a reddish jelly-like mass, leav-

ing a colorless liquid, the serum, above and around the clotted mass of corpuscles and fibrin.

Quicker results may be secured by means of a beating arrangement of twisted wires, extending down into the bottle, which is placed in it before it is sterilized. After the blood is collected this beater is twirled by means of the handle extending through the neck of the bottle. This motion hastens the separation of the fibrin, a large mass of stringy fibrin collecting on the beater, and enmeshing most of the white and red corpuscles.

Up to this point it is relatively easy to handle the blood aseptically and maintain its sterility. Filtration through the best types of filters is often added, and if further treatment is necessary to insure sterility, disinfectants may be added. Preference is often given to such volatile substances as chloroform which evaporate and do not, therefore, complicate matters by introducing salts, etc., into the blood. Ultra-violet rays are recommended by some because they, too, add nothing to the serum. When as described in the next paragraph the antiserum is still further modified, the treatment (filtering, adding chemicals) to insure sterility is deferred until such modification has been completed.

Modified Serums.—The preparation of most antisera—practically all with the exception of diphtheria—stops at this point. In all cases where it is practicable, however, further treatment should be undertaken to concentrate the antitoxin and thus lessen as far as possible the unfavorable effect produced by the introduction into the body of foreign proteins from another individual. There is a loss of “protective principles” during this process which prohibits such modifying or concentrating processes with almost all serums, diphtheria antiserum being the outstanding exception. One form of tetanus antitoxin may be somewhat modified, but tetanus antitoxin in common with other antisera is generally used as “straight” antiserum. (See also *Antibody Extracts*, p. 71.)

(3) **Further Modification of Diphtheria Serum.**—The antitoxic qualities of this antiserum are very intimately associated with the globulins (euglobulins, pseudoglobulins) in the serum. Although Hantoon has recently shown (See p. 73) that antibodies themselves are not euglobulins or pseudoglobulins, it is neverthe-

less necessary in the ordinary modifications of antiserums to keep this association in view; and in each process of the preparation of diphtheria antitoxin, such as filtering, the worker must retain the part which contains the globulins, especially the pseudoglobulins. In some methods there are several different filterings, the desired pseudoglobulins being sometimes in the liquid filtrate,

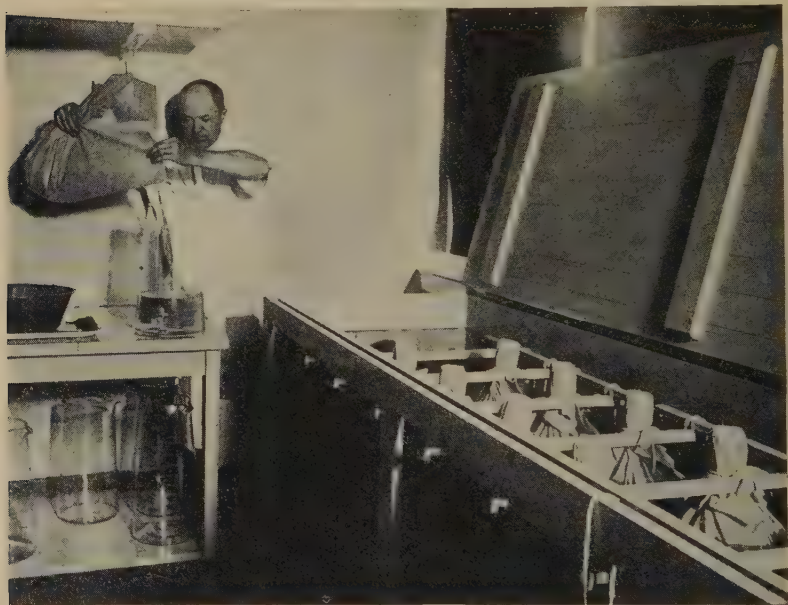


FIG. 67.—At the right, dialyzing the antitoxic globulin in running water to separate from it the inert soluble salts. At the left, one of these parchment bays, through which water has passed during its stay in the running water, is being emptied into a receptacle, previous to the final filtering and bottling. H. K. Mulford Company.

sometimes in the solid precipitate. The recent method described below is one of the simplest.

The immune serum is diluted with an equal amount of water and a very large amount of ammonium salt (ammonium sulphate) is added until the material is about one-third saturated with the ammonium sulphate. This mixture is then heated (61°C.) for two hours, and filtered through filter paper. This leaves the antitoxin globulins in the precipitate. The liquid filtrate is therefore discarded and the grayish-white precipitate

scraped off the filter paper and compressed between many thicknesses of filter paper until most of the liquid is absorbed. This also removes much of the salt which was used to precipitate the antitoxin. The rest is removed by dialyzing, the nearly dry precipitate being placed in a large parchment bag (Fig. 67) and suspended in running water (flowing tap in a sink) for several days, during which time the salts are dialyzed out through the parch-



FIG. 68—Drying the precipitate containing the antitoxic globulins, by pressing out the water. The precipitate is placed between many thicknesses of absorbent filter paper: several lots of such precipitate (separated by boards) may be pressed nearly dry in the same press as shown in the accompanying illustration. H. K. Mulford, Co.

ment. Meantime some water passes through into the parchment bag, and the putty-like precipitate gradually changes to a brownish liquid, the modified anti-serum or the antitoxin. (This antitoxin may be still further diluted with water, and salts like those in human blood added to make it similar to the blood in salt content [isotonic].)

Owing to the long period of treatment just described it is now necessary to make sure that no micro-organisms are present in the finished antitoxin. Sterility is obtained (1) by using such disinfectants as the cresol compounds or chloroform and (2) by filtering. Paper pulp, glass wool, etc., may be used to remove

the coarser debris, after which the filtration is completed by means of a Berkefeld filter. The antitoxin is now free from micro-organisms and also from shreds of fibrin and broken cells which might cause death if they passed into the finer capillaries.

This modified antiserum retains about 70 per cent. of the original antitoxic power of the original whole serum. Although some of the antitoxic power is thus lost, the resulting liquid is so concentrated that a cubic centimetre of the original blood may be represented by but $\frac{1}{4}$ or $\frac{1}{6}$ of a cubic centimetre. Antitoxin given in this form, therefore, contains much less of the various serum or blood proteins per cubic centimetre, and is therefore less irritating when injected.



FIG. 69.—Intravenous injection of drugs, serum, etc., is done as shown here. In subcutaneous or intramuscular injection, no effort is made to strike a blood-vessel. THOMAS AND IVY, Applied Immunology. J. B. Lippincott Co.

This antitoxin is stored aseptically in large sterile bottles until its strength can be determined. It is then put into small bottles, or preferably into syringes ready for individual use, each bottle or syringe containing a little more than the usual protective dose. The amount is indicated by a label, which gives the total value contained in the bottle or

syringe, and often also a graduated scale for convenience in measuring the proportion of the contents given a patient. If kept sealed in a cool dark place diphtheria antitoxin can be kept for months without appreciable loss of strength.

Standardization of Diphtheria Antitoxin.—The strength of the antitoxin secured from the horse is determined by finding how much of it is needed to protect a medium-sized guinea pig against a fatal dose of diphtheria toxin. When that amount is determined, it is used as a basis for estimating how

much is necessary to protect human beings. For such a large animal as man several hundred, or even thousand, times as much are necessary, as there is so much more tissue throughout which the antitoxin must be distributed to make sure no part is left unprotected against the diphtheria toxin, which is being rapidly distributed by his circulating blood from the infected area in his throat.

The first step in the process of determining the strength of a given antitoxin consists in placing in each of a series of about ten test tubes just enough diphtheria toxin to kill a standard-sized guinea pig (250 grams) on or by the fourth day. This is often spoken of as the minimal lethal dose (M.L.D.) and may be as small an amount as $\frac{5}{1000}$ of one cubic centimetre, a very small amount indeed. To each of this series of tubes is added progressively increased amounts of the antitoxin to be standardized. The combined contents of each tube is then inoculated into a different guinea pig, each properly labeled to correspond to the tubes so that we can tell by the fate of the guinea pigs exactly where in the series the antitoxin just neutralized the toxin. All the guinea pigs having enough or more than enough antitoxin will recover. (Care is taken, of course, to use healthy guinea pigs of the same relative size, vigor, etc.)

This amount of antitoxin that will protect a guinea pig is much too small for a workable unit (speed and accuracy of measurement, etc.), and since man will need so much more antitoxin than that minute amount we use one hundred times the guinea pig amount as a "unit" of diphtheria antitoxin.* Even this makes too small a bulk for a satisfactory working basis, for horses may yield one thousand or more such units of antitoxin to one cubic centimetre of blood, though 800 units is considered high. (See p. 76.)

Dosage.—The amount of diphtheria antitoxin given a person varies with such conditions as the age or size of the individual, the stage of the disease, and its relative severity; and the dose also varies in size, depending upon whether it is given to aid an individual actually ill with the disease or merely to prevent him from contracting the disease.

* A unit of tetanus antitoxin is 1,000 times the amount needed to protect a large guinea-pig (350 gm.) against one fatal dose of tetanus toxin.

As a preventive only, the usual dose varies from five hundred to one thousand units for an adult; this gives an immunity lasting about two weeks, though it may last 30 days. In epidemics, the fact that injected antibodies disappear rather rapidly from the body makes it advisable to repeat the treatment for nurses or others subject to re-exposure every 10 to 14 days until the danger is past. (See p. 201.)

In treating actual cases of diphtheria the dosage is larger, especially if treatment has been delayed. A delay of even a few hours only may be serious, for toxin makes very stable combinations, and once it has combined with the tissues, the results cannot be neutralized by increasing the amount of antitoxin given. The amount of antitoxin given varies also with the method of administering it—intravenous injection being 500 times as effective as subcutaneous injections would be, consequently the latter method is no longer recommended. Doses usually vary from 5,000 to 10,000 units when given on the first day of the disease; in the very severe attacks 15,000 to 20,000 units might be used, but extremely large doses (100,000 units) are not now considered advisable; if beneficial results with the smaller doses are not evident in a short time (eight hours) the treatment is repeated.

Preventive Treatment Using Toxin.—The protection obtained by injecting a toxin is due to an active immunity produced in response to toxin stimulation. This is, of course, essentially the same process as when the results are obtained by giving an ordinary vaccine made of living or dead bacteria, and therefore protection against diphtheria by the injection of toxin would be properly discussed under vaccines, page 174.

This toxin treatment, however, is now commonly spoken of as a *toxin-antitoxin* treatment; and since the word antitoxin in this connection always raises a question in the student's mind, it is described here to explain the antitoxin part of the procedure.

On page 91 note that the horse was not given toxin only—but a combination of toxin and antitoxin, for the reasons stated there. Students often find this difficult to understand. What, they say, is the use of adding antitoxin if it only neutralizes part of the toxin particles—why not give only the toxin represented by the difference, omitting the antitoxin altogether?

Here we come to one of the weak places in the receptor theory

—of which the student was warned. According to Bordet, the combination of toxin and antitoxin is probably more like the well-known combination seen when starch and iodine are mixed together. A large amount of iodine can be used, combining with each starch grain, and making the grains blue black in color. If less iodine is used each grain combines with less iodine, becoming merely blue in color; if still less iodine is used, each grain again has correspondingly less iodine combining with it and becomes only light blue or pale lavender in color.

In toxin-antitoxin mixtures, the antitoxin seems to combine with toxin in this same way, each toxin particle being slightly modified by the relatively small amounts of antitoxin present (See Fig. 88?); therefore, in a toxin-antitoxin mixture in which the antitoxin is not great enough to neutralize all of the toxin, the toxic effects are due not to a small unneutralized or uncombined excess of the original toxin but to the very large amount of weakened toxins at work. It is possible—to carry on our illustration—to use so little iodine or so much starch that some of the starch grains are wholly unaffected. This would be true with toxin and antitoxin combinations, of course, and in such treatment the relative amounts of toxin and antitoxin given must be very accurately determined; with too little antitoxin the individual is not properly protected, and with too much antitoxin, the mixture is too inert—not sufficiently irritating to arouse the active immunity responses for which it was given.

This toxin-antitoxin treatment, it is evident, causes the individual to make his own protective substances just as the horse does, and is used only where it is probable that there will be sufficient time for the individual to make his own antibodies. In actual illness we cannot wait for the individual to develop active immunity, and therefore passive immunity is secured by giving antitoxins. In sudden outbreaks the children or other individuals directly exposed should receive antitoxins, the toxin-antitoxin treatment being given them later when the immediate danger of direct infection is at an end. Those less directly in contact with the first case or cases will, as implied in the beginning of this paragraph, be given the toxin-antitoxin treatment.

When time is not an important consideration it is much cheaper and much simpler to have each individual make his own

antitoxins, for all that is needed is to inject into each individual a little diphtheria toxin, using with it but a fractional amount of the expensive antitoxin that would otherwise be required to give passive immunity. This is not only cheaper, but infinitely better in every way as the passive immunity conferred by antitoxin lasts but two to four weeks only, whereas the active immunity resulting from toxin-antitoxin treatment persists for years and perhaps for life.

The Schick Test.—It is possible to determine by a skin test whether or not an individual is susceptible to diphtheria—whether or not he has antitoxin in his blood that will protect him against an attack of that disease. This test, which is called the Schick test (Pl. 1) is made by injecting a tiny amount of diphtheria toxin (about one-tenth of a cubic centimetre, or more definitely, $\frac{1}{50}$ th of a minimal lethal dose for a 250-gram guinea pig) beneath the outer layer of the skin of the forearm. If there are no antitoxins present to neutralize the toxins injected, the tissues round about will be irritated by the toxins as shown by the formation of a red, inflamed area (Plate I), which appears in 12 to 48 hours, and is at its height in three to four days; this lasts from one to two weeks, and as the inflammation gradually disappears, there is a superficial scaling and a characteristic browning of the area. But if the individual is immune to diphtheria the antitoxin present in his blood will neutralize the injected toxins before they can so affect the tissues, and the characteristic swollen red area will not be formed; we say, therefore, that this individual gives a negative Schick test, indicating that he is immune to diphtheria.

Pseudo-Reactions in the Schick Test.—In order to interpret correctly the result of the Schick test it is necessary to differentiate carefully between a true Schick reaction and the false (or non-specific protein) reaction which appears in some cases. This pseudo-reaction seen in persons unusually sensitive to the irritating effect of foreign proteins develops in consequence of the minute amount of foreign protein necessarily introduced with the toxin. The pseudo-reaction may appear alone or in combination with the Schick reaction, and it is entirely without significance except as a possible source of error in interpreting the Schick test. (An irritation due to proteins as such and not to the diphtheria toxin may be mistakenly interpreted as due to the toxin.) To

guard against this danger a control test should be made on the opposite arm at the same time that the Schick test is made, using for this control diphtheria toxin which has been heated to destroy its toxin content, leaving the inert protein as the only irritating substance present.

Value of the Schick Test.—The Schick test, it can be readily seen, is a very helpful one. In epidemics it enables one to pick out promptly the susceptible children or nurses and other attendants and protect them by giving them antitoxins—or if time allows, the toxin-antitoxin treatment—thus making the control of diphtheria much more possible. Since many people are immune to diphtheria, it also enables us to save large sums of money by determining which individuals do not need treatment, variously estimated as 30 to 50 per cent. of babies under two, 40 to 70 per cent. of the children from 2 to 4 years, 45 to 75 per cent. from 4 to 6 years, 45 to 80 per cent. from 6 to 12 years, 80 to 85 per cent. from 15 to 18 years, 85 to 95 per cent. of adults. Zingher states that in institutions and schools the susceptible children may be but 20 to 25 per cent. of the total.

Recent tests with large numbers of public school children in several eastern cities have indicated that 30 to 80 per cent. of the various school populations were susceptible to diphtheria. Without a word of explanation such a wide range in susceptibility might seem to indicate a lack of reliability in the Schick test. Age differences as shown by the figures in the preceding paragraph enter in; the proportion of girls and boys also affects the total percentage for the group, as girls are more susceptible to diphtheria than boys. An analysis of the Schick results has shown, however, an interesting relationship between the percentage of susceptible children and the general social status of the group tested. In New York City institution and school groups from the poorer and more crowded areas showed 10 to 20 per cent. susceptible children. In other more favored areas the per cent. of susceptible children was higher, representative schools containing 29 per cent., 41 per cent. and even 67 per cent. susceptible children. In three private schools of New Jersey the Schick test showed the presence of 73 per cent., 79 per cent. and 85 per cent. susceptibles, respectively. Children subject to constant or repeated exposure apparently develop a natural immunity.

The high susceptibility in the urban schools and neighborhoods is explained on the basis of a segregation of the children remaining susceptible because protected from exposure.

Toxin-Antitoxin Treatment of Infants.—Since children between one and five are more susceptible to diphtheria than any other age group (80 to 85 per cent. of the cases and deaths), immunity should be developed as early as possible by giving the toxin-antitoxin treatment to all children yielding a positive Schick test. It was at first thought that this might be done advantageously by giving the treatment during the least susceptible part of the infant's life—the first six months. Later work with several thousand infants has shown, however, that the toxin-antitoxin treatment has very little effect upon very young infants, as the presence of the antitoxin derived from the mother prevents or limits the formation of such antibodies in the child. This inherited immunity (85 per cent. of the newborn give negative Schick tests) is usually lost during the first six to nine months, though it may persist six to nine months longer. By the second year, therefore, children should be actively immunized against diphtheria by the toxin-antitoxin treatment, thus forestalling the possibility of infection during the most susceptible part of the pre-school period.

Results of Toxin-Antitoxin Treatment.—The results are most satisfactory, both with regard to the higher degree of immunity developed and the total lack of untoward results, even where many thousands of children have been so treated, as in New York City. The doses are small in bulk, consisting of one or two, or less often, three injections of one cubic centimetre of the toxin-antitoxin mixture given subcutaneously in the arm. The mixture is so slightly toxic that when five cubic centimetres are given a guinea pig there results a local induration at the injection site, which is followed by a later paralysis, but never by the "acute death" of the animal.

Immunity develops in three to twelve weeks, varying with the dosage—that is, with number, size, and proportions of toxin-antitoxin doses. If individuals do not yield negative Schick tests after the first treatment, full immunity is usually developed by a second series of treatments. As high as 85 to 100 per cent. im-

munity has been obtained with a single series of injections. In one institution of over 1,000 children 81 per cent. gave negative Schick tests after single injections.

The immunity thus developed is quite lasting. Retests on thousands of children in New York City have shown the immunity still present after 3, 4 and even 5 years, and experienced workers in this field feel that the immunity thus developed will probably persist throughout life, and that there is every hope that diphtheria may be eradicated as completely as smallpox.

Diphtheria Antitoxin.—During the period of forty years that diphtheria antitoxin has been in use there has been more or less discussion of the ill effects following its use. These reports of ill effects have been greatly exaggerated. According to Park, in 140,000 cases so treated in New York City, there have been but two deaths which were due to the antitoxin; and only about one in 10,000 treated individuals develops alarming symptoms.

Disturbances following the use of diphtheria antitoxin are due entirely to the effect of the foreign protein in the horse serum and not to the antitoxins it contains. For "serum sickness" and the occasional ill effects of antitoxins as they are prepared to-day see the chapter on Anaphylaxis.

Diphtheria antitoxin has reduced the mortality from diphtheria at least 60 per cent. (Fig. 70). In the pre-antitoxin days, according to Zingher, the mortality was 70 to 75 per cent.; with the introduction of antitoxin in 1894 the mortality has gradually been reduced to 10 per cent., a more or less constant figure for the past eight to ten years. Diphtheria antitoxin has been called "a specific and sovereign remedy," and deservedly so, for if used during the first twenty-four hours of the disease it is almost uniformly successful. The persistently high mortality of 10 per cent. is explained as due to "delayed application for treatment on the part of the patient, delayed recognition of the disease on the part of the physician, or to both of these factors."

Tetanus Antitoxin.—Tetanus antitoxin is, as described on page 93, usually whole serum, though a modified (or globulinized) serum is also commercially available. Tetanus antitoxin is produced in horses, which are injected much as is the case with the diphtheria antitoxin horses, with gradually increasing doses, until

in a few months the horse is being given 100 c.c. of strong tetanus toxin, a surprisingly large amount when one considers that tetanus toxin is one of the most poisonous substances known— $\frac{6}{1,000,000}$ of a gram killing a large (350 gram) guinea pig. Be-

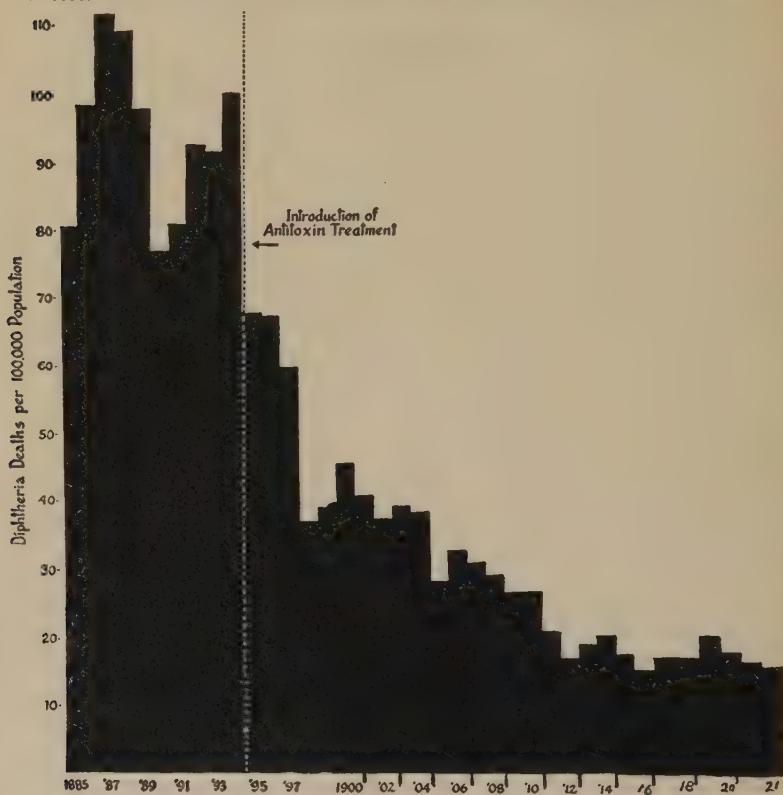


FIG. 70.—The effect of antitoxin upon the diphtheria death rate in New York State. *Health News*, 1914, completed through 1921 by information supplied by New York State Department of Health.

cause tetanus toxin is such a strong poison, horses are not bled until at least two weeks after the last injection of toxin, so as to make sure that all the injected toxin has disappeared from the blood and cannot therefore be transferred to man.

Treatment.—Since tetanus antitoxin is absorbed slowly, and since, to be effective, it must reach the central nervous tissues, it

is better to give it intraspinally or at least intravenously, in treating actual cases of infection.

At present tetanus antitoxin is used mostly as a preventive, in the treatment of wounds which are likely to contain tetanus organisms. In treating such wounds two things must be kept in mind: (1) that the intestines of the larger domestic animals harbor tetanus organisms, and that, therefore, street, road and farm soil is very likely to contain tetanus spores; and (2) that the tetanus organisms grow best where little oxygen is present, and that spores left in the recesses of jagged wounds made by explosives (fireworks, explosives used in modern warfare) are often sealed up in such recesses by the clotting blood forming little anerobic pockets favorable for their development. The present low rate for tetanus following Fourth of July accidents, and the relative absence of tetanus in the Allied forces in the recent war are both due to the early or prompt use of tetanus antitoxin. As a matter of routine, soil-infected wounds were treated with antitoxin, at least 1,000 units (See p. 97) being injected in the wound region. Since tetanus antitoxin disappears quickly from the body (8-10 days), a second treatment is given at the end of a week, and another usually during the third week.

A very large part of the tetanus antitoxin used abroad during the first part of the recent war was made in the United States, some by commercial laboratories, but a very large part by the Department of Health Laboratories of New York City. Soon, however, hundreds of horses were manufacturing tetanus antitoxin on the other side of the Atlantic, using for the purpose the more productive tetanus cultures sent by the New York City Department.

In ordinary civil practice, where wounds are usually more promptly cared for, the preventive treatment, 1,500 units, is usually given subcutaneously or intramuscularly.

Antitoxin against Gas Gangrene.—Occasionally soil-borne organisms other than tetanus cause serious wound complications. One of the worst offenders is Welch's bacillus (Fig. 71), or the gas gangrene bacillus, which forms a virulent toxin or poison, destroying muscle tissue and causing a gangrenous condition of the area infected. Against this organism, an antitoxin (anti-

serum) has recently been prepared which has been successfully used in many cases. Where wounds are infected with other septic organisms (malignant edema organism, Fig. 52; Pasteur's "anthrax septicæ") the appropriate antitoxin should be used. Unfortunately infections are not always due to a single type of such organisms. Besides, various aerobic organisms, such as hemolytic streptococci, are often important disintegrating or toxic agents, and their presence often adds greatly to the difficulty in securing effective antiserums for treatment.

The so-called antitoxins used in this connection are anti-serums—whole serums; and are given in preventive work much as tetanus antitoxin is used—intravenously and also in frequent injections around the wounded area.



FIG. 71.—The gas gangrene bacillus.
1880. Bull.

In treating infected war wounds, more use than usual was made of the microscope findings, for the microscope was used not only to aid in determining the type or types of organisms present, but to show the progressive changes in the infected areas; these findings were made not only to determine the subsequent antiserum dosage, but to indicate when such areas were sufficiently free from organisms for open wounds to be closed with safety.

The relative infrequency of such wound infections in civil life and the variety of organisms related to all such infections make it unlikely that we will ever have—in this connection—anything like the satisfactory results now obtained with diphtheria antitoxin.

Other Antitoxins.—Antitoxins against botulism (See p. 89) have not been put upon a successful basis.

Shiga's dysentery bacillus (Fig. 52), the most toxic of the dysentery and para-dysentery group, forms a toxin against which

an antitoxic horse serum is produced, which has reduced the mortality of that type of dysentery in Japan from about 30 per cent. to about 10 per cent. While Shiga's dysentery is less common in the United States than in Japan, it is one of the two most common

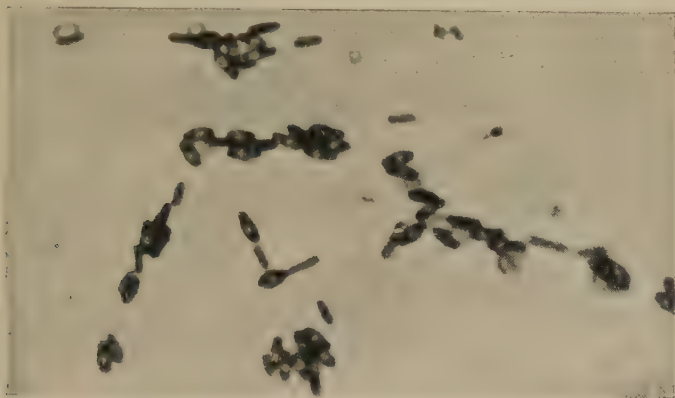


FIG. 72.—A three-day culture of malignant oedema organisms; most of the spores are centrally placed, causing a bulged appearance. ADAMSON, *Journal of Pathology and Bacteriology*. (Photograph by C. Powell White).

types of dysentery here; there have been recent indications of an increase in this type of dysentery, especially in the southern States.

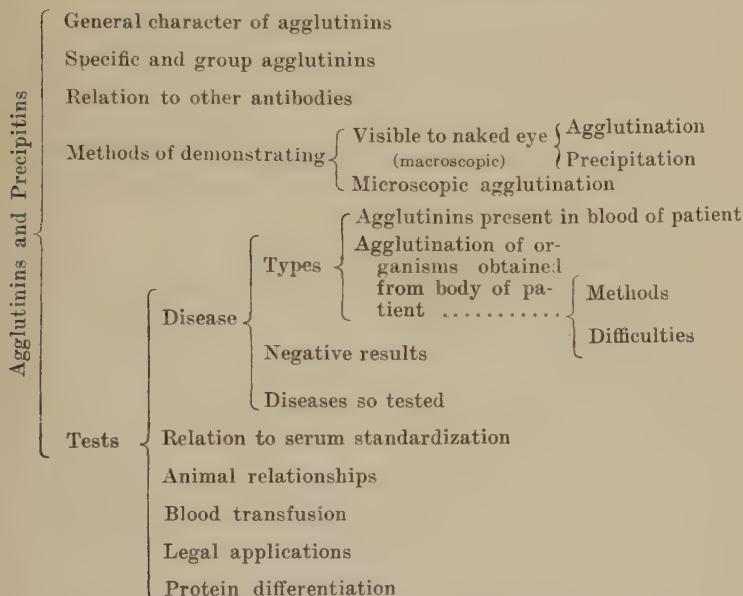
STUDY SUGGESTIONS

1. What are toxins?
2. How do old or changed toxins affect the strength or toxicity of a toxin? How would the presence of toxoids in toxin preparations affect the determination of the strength of an antitoxin?
3. Name three diseases which are characteristically toxin diseases, giving one definite associated effect or symptom for each.
4. Describe the method of producing diphtheria antiserum in horses.
5. Describe one preparation used in treating disease in which immune blood undergoes special treatment—something more than clotting and drawing off of the serum.
6. Give two ways in which immune serums may be made sterile without any attendant formation or retention of solid particles.
7. How is diphtheria antitoxin standardized?
8. Describe the Schick test.
9. Describe the toxin-antitoxin treatment for diphtheria.
10. Write in semipopular form (for parents of school children) a statement presenting the advantages to be gained by the toxin-antitoxin treatment against diphtheria.

11. In a family containing two young children who slept together and a third older child who was away on a visit, one of the younger children "came down" suddenly with diphtheria. What treatment would you advise for each? If the absent unexposed child returned during the illness, what protective or preventive treatment should be given him?
12. In the City of Washington there were one year 143 cases of lockjaw or tetanus from Fourth of July wounds; in the ten years following when the sale of fireworks was prohibited no cases developed following the Fourth of July celebrations. Explain the difference in these figures, including in your answer the probable relationship of (1) the actual number of surface or skin breaks made on the Fourth, (2) the type of wounds formed, and (3) the substances gaining entrance into the body.
13. If the blood taken from a horse shows 700 diphtheria antitoxin units to a cubic centimetre of serum, and if the modified form of the serum (described on page 93) retains 70 per cent. of the original antitoxic power, how many cubic centimetres would have to be injected into a patient needing 10,000 units?

CHAPTER V

AGGLUTININS AND PRECIPITINS



Although bacterial agglutination by the blood (or serum) of immune animals has been known for about thirty years, it is only recently that it has been positively demonstrated that such agglutination really takes place in the body (Fig. 73) and is not merely a laboratory phenomenon. And although agglutination tests have for years been a routine laboratory procedure of undeniable value in diagnosing disease, especially typhoid, it is only within the last few years that we have had incontrovertible evidence that agglutination is a most important preparatory step in the destruction and in the elimination of bacteria from the body.

According to Bull, "in order that the bacteria may be promptly removed from the blood stream it is requisite that they be first agglutinated, which condition is also required in order

that they be destroyed *en masse* within the organs, a process achieved, apparently, chiefly through phagocytosis."

Bacterial Agglutination.—When a loopful of bacteria, of a given kind such as cholera, is properly diluted and mixed with a drop of the blood of an individual recently recovered from that disease, the bacteria show two distinct changes: (1) All movement ceases—true motility as well as Brownian movement; and (2) all the organisms stick together in clumps or small masses, as if they have become sticky or glutinous, hence the term, agglutination.

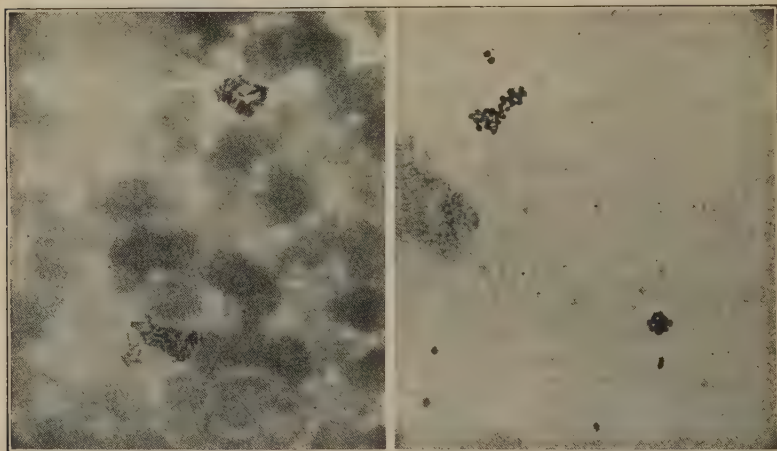


FIG. 73.—These photographs show that agglutination is not merely a laboratory phenomenon but actually occurs in the animal body. A rabbit previously made immune to typhoid by injections of *Bacterium typhosus* was later given a heavy injection of the same organism. The left photograph of blood taken from the heart 30 seconds later shows clumps of bacteria and indicates how rapidly agglutination took place. The agglutinated cocc in the photograph on the right were from the blood of a rabbit treated similarly with pneumococcus organisms. Photograph, Bull; BROADHURST, Home and Community Hygiene.

This clumping or agglutination is not a vital phenomenon or response because it takes place with dead as well as live bacteria. It is a delicate phenomenon occurring in very weak dilutions, and the real cause is far from clear. It may be hastened or inhibited by very slight chemical differences, such as variations in the amount of salts, acids or alkalies present, and is usually vaguely described as a "chemical-physical process involving the bacterial cell and an agglutinating factor present in the serum," or, somewhat similarly, as an electro-chemical phenomenon.

Agglutination itself does not kill bacteria, though it does lead to their destruction, because these agglutinated clumps do not make their way so readily through the tiny capillaries, and are, also, filtered out or held back in such glands as the lymph nodes and spleen, being therefore, much more rapidly captured and destroyed by the white corpuscles.

Agglutination a Specific Phenomenon.—One of the oldest facts known about these agglutinating substances or agglutinins is that they are specific; for example, the blood of a person having typhoid fever will agglutinate typhoid bacteria and not cholera organisms; while the blood of a cholera patient will agglutinate cholera bacteria, and not typhoid.

This specificity makes it possible to diagnose disease by testing for agglutination. If the bedside or clinical symptoms of the patient indicate typhoid fever, the diagnosis can be made certain by showing that the blood of the patient agglutinates typhoid bacteria. (See p. 114.)

Group Agglutinins.—While this action is specific, related organisms, such as the various typhoid types, are enough alike to respond

in a general way to the agglutinins made against any one of the related group. For example, in paratyphoid fever due to the so-called paratyphoid B organism (Fig. 74), the blood of the patient not only agglutinates the paratyphoid B organism, but may also agglutinate paratyphoid A and ordinary typhoid as well. (See also p. 112.)

This group relationship does not, however, make diagnosis impossible, for though as described in the paragraph above the blood of the paratyphoid B patient may agglutinate all of the three types of bacteria, it does so in varying degrees; this blood

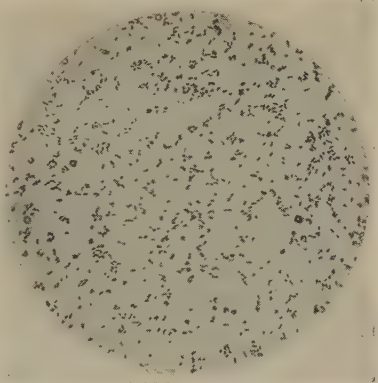


FIG. 74.—Para-typhoid B bacteria broth culture (x 700). Sands.

may agglutinate the "paratyphoid B" organism although it is diluted as much as 5,700 times, but it will not certainly agglutinate typhoid organisms in dilutions of more than 120, or "paratyphoid A" in dilutions of more than 10. Similarly, the blood of an individual immune to typhoid may agglutinate typhoid bacteria in dilutions of 10,000 or more, while the dilutions causing the agglutination of related organisms may be as low as one to 1,000 for the paratyphoid organisms, or one to 100 for the colon bacterium.

There are occasional exceptions to these differential results; *e. g.*, the serum of a horse immunized to one of the dysentery organisms also possessed the power of agglutinating the colon bacillus when diluted 10,000 times. Less often still such excep-

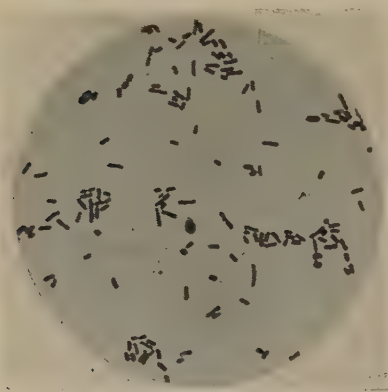


FIG. 75.—*Bacterium coli*, an organism common to the intestinal flora of man and the larger domestic animals. Williams, Brown and Earle.

tions may be found as the agglutination of unrelated organisms, illustrated by the agglutination of a variety of *Proteus vulgaris* (*B. proteus vulgaris*) (Fig. 76), by the blood of typhus fever patients. (See p. 123.) Such exceptions are, however, not common, and diagnosis based on agglutination can safely be made by using dilutions higher than the group or cross-agglutination strength, *e. g.*, dilutions above 1,000 for the typhoid serum described in the preceding paragraph.

The agglutinating action of immune blood or serum is remarkably prompt and vigorous. That, in fact, is one reason why experimenters did not earlier demonstrate that agglutination does occur in the body. The second reason is that the white corpuscles take in and destroy these clumps with remarkable celerity, and therefore the agglutinated clumps as such were not found by workers, because they waited too long (hours or even days) for the clumping to take place before drawing blood for examination.

The accompanying photograph (Fig. 73), shows typhoid bacteria clumped by the blood of a rabbit which had been made immune to typhoid by several earlier injections of typhoid bacteria; after immunity had thus been established, the rabbit was injected with a heavy dose of typhoid bacteria which were immediately agglutinated as shown by this photograph of blood drawn thirty seconds after the bacteria were injected.

No practical modification or concentration of antiserums containing agglutinins has yet been made for therapeutic work, although the agglutinins may be present in almost unbelievable amounts. (See, however, p. 124.) This remarkable agglutinin content of immune blood can be illustrated in several different ways. For instance, one cubic centimetre of pneumococcus antiserum can agglutinate ten million bacteria. The antiserum obtained from animals immunized against the Shiga dysentery bacteria may be active when diluted at least 20,000 times. Recently Bull showed with a horse immunized against typhoid that one drop of the horse serum could agglutinate all the billions of typhoid bacteria grown on four agar slants even when they were suspended or diluted in a whole litre of salt solution.

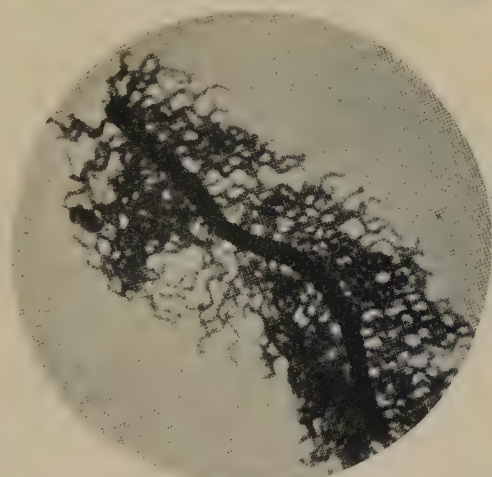


FIG. 76.—*Proteus vulgaris*, much enlarged. (x 2000) showing incidentally the flagella which give the power of independent movement. Many of the other rod and spiral organisms pictured in this text have similar appendages, but they have not been stained with the special technic necessary to show the flagella. VINCENT, *American Journal of the Diseases of Children*.

Agglutinins Not Related to Lysins.—While the action of agglutinins is a preliminary step in the destruction of bacteria by the white corpuscles, these agglutinins are not to be confused with lysins (See p. 49) which acting alone can dissolve and destroy

bacteria. Agglutinins may exist in an antiserum independent of lysins, and lysins independent of agglutinins, or both may be found in the same antiserum.

Visibility of Agglutination Outside the Body.—Usually, as in typhoid agglutination, the clumps are not distinguishable without the aid of a microscope, and the mixture of

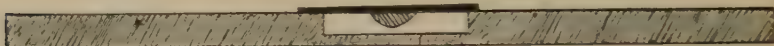


FIG. 77.—A hanging drop, showing the drop suspended in the chamber under the coverglass; to keep the drop from drying up, the contact surface of cover glass and slide are sealed with vaseline.

antiserum and bacteria is made as a hanging drop (Fig. 77), as described on page 110 and page 118, and examined with the high power of a microscope (Fig. 78).

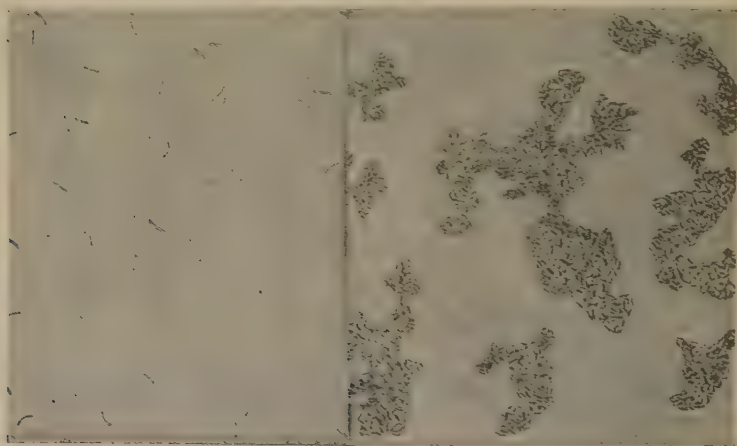


FIG. 78.—Left: A hanging drop showing single free-swimming typhoid bacteria. Right: the same, except the addition of serum of a typhoid patient has caused the agglutination of the typhoid organisms. THOMAS AND IVY. Applied Immunology. J. B. Lippincott Co.

Sometimes, however, as with meningitis, the clumps of bacteria are large enough to be seen with the naked eye, and the mixture is made upon an ordinary flat microscopic slide, which is then held between the eye and the light, to enable the worker to distinguish the whitish "flakes" or agglutinated masses. This

slide-agglutination may also be demonstrated with typhoid, cholera, paratyphoid and dysentery organisms.

In some cases, such as glanders, the results are more easily determined when much larger amounts of each material are used; test tubes containing the antiserum and bacteria are examined for turbidity changes and the final flaking or precipitation of the bacteria as they fall out of the solution to the bottom of the tube. (See Fig. 79.) This precipitation and the resultant clearing of the tube contents is essentially the same as the agglutina-

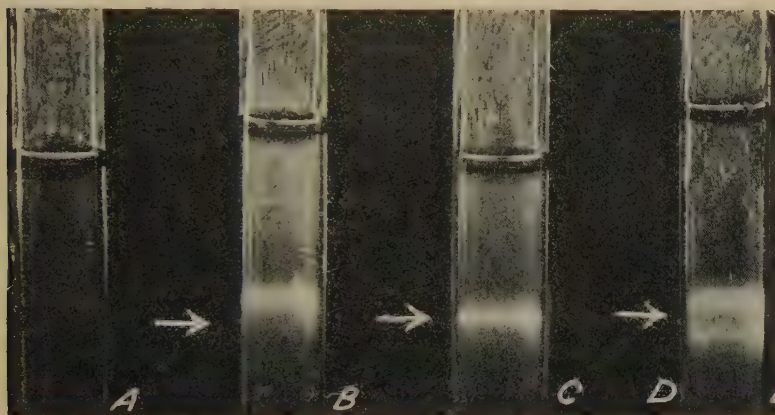


FIG. 79.—Precipitin test for glanders. Serum from a normal horse was added to the glanders bacteria suspension in tube *A*, negative reaction. Positive results are shown in *B*, (occult glanders) *C* (nasal glanders) and *D*, (chronic farcy or glanders), where as indicated by arrows, a cloudy ring is seen at the point of contact of the bacterial suspension and the serum. MOHLER and EICHORN Bureau An. Ind., U. S. Dept. Agriculture.

tion observed in the minute amounts used on microscope slides. As used in routine test work it may be a much slower process, however: one to three hours in the precipitin test for typhoid, and seventy-two hours in the usual routine precipitin test for glanders.

Precipitins.—The action of agglutinins and precipitins is essentially the same; the effect on large particles, such as bacteria, is visible as agglutination, and the effect on small particles, such as colloid particles or proteins in solution is indicated by the resulting clouding and precipitation. Precipitins like agglutinins are specific; a precipitin formed against a given protein, such as human milk, for example, causes precipitation with human milk

and not with other proteins or even other kinds of milk, such as cow's milk.

While the principal theories and procedures accepted for agglutinins are applicable to precipitins, there are, nevertheless, important differences which can not be completely described here. The most important for our purposes are the two following: (1) To demonstrate precipitins we must be able to secure a clear fluid containing the related protein; this is not always possible, and limits decidedly the number of diseases in which precipitin tests can be made. (2) Another reason that precipitins are less used in diagnosis is that precipitins are not easily demonstrated

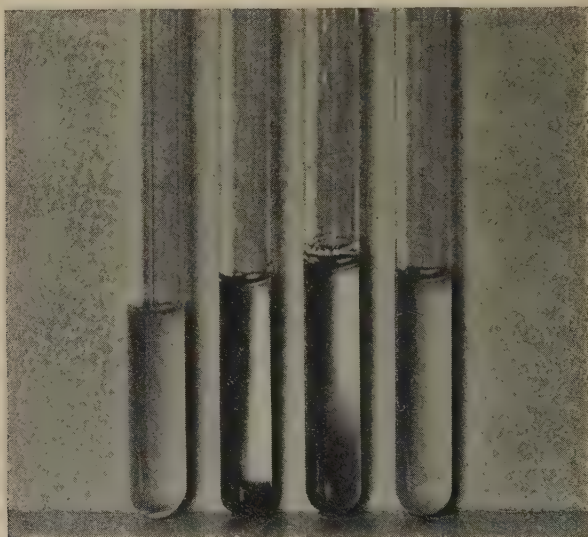


FIG. 80—A series of tubes showing precipitin reactions against pneumococcus organisms; left negative; to right, varying degrees of precipitation with varying dilutions of immune serum. H. K. Mulford Co.

in the serum of the sick, being apparently a much slower response than the formation of other antibodies.

When demonstrable this precipitating effect may be observed with bacterial extracts as well as with whole bacteria. It is commonly obtained with "unformed proteins" or non-cellular proteins, such as milk or serum. For further discussion of precipitins see Fig. 84 and page 126.

Tests Based on Bacterial Agglutinins and Precipitins.—

Tests for agglutinins (and precipitins) may be used to show several different things: (1) That an individual is developing a given disease, having already begun to form agglutinins against that particular species of bacteria; there may be a gain of several days by using this method of diagnosing a disease. (2) Agglutinin tests are also valuable during the active stages of a disease, particularly in cases where the disease develops atypically, as often occurs in typhoid, thus delaying the possibility of diagnosis by the usual clinical evidences. (3) Agglutination tests are also made to identify the type or variety of organism causing the disease. At

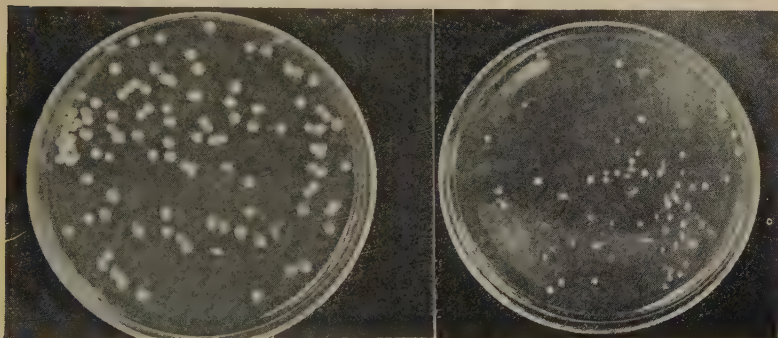


FIG. 81.—Plates showing typhoid colonies. A diluted sample of human feces was added to these two plates, containing the usual agar plus a dye, brilliant green, which allows typhoid to develop while inhibiting most of the other intestinal bacteria. Even in these some of the inhibited effects may be seen, as the typhoid colonies are much smaller in the right hand plate containing the larger amount of green dye. Photographs by Heins from plates made by the Bureau of Laboratories, Health Department of the City of New York.

least four distinct types of pneumococcus organisms are recognized in pneumococcus infections. If an antiserum is to be given, it should be an antiserum made in response to and against that particular type of pneumococcus. (See p. 80.) (4) In carriers, such as typhoid carriers, the immunity and continued good health of the carrier is no doubt partly due to the antibodies he continues to produce during the persistence of the typhoid bacteria in the intestinal tissues, bile duct, etc. Suspected carriers may be cleared—or the suspicions concerning them may be confirmed—by such agglutination tests, even months or years after apparently complete recovery (See p. 56).

The tests (See also p. 121) involving the use of specific agglu-

tinins or proteins are of two different kinds: (1) In the first kind the individual's blood is examined to see if it will agglutinate or precipitate a known species of bacteria. The presence of such reacting substance in his blood is taken as evidence that the specific causal organisms are or have been present and are responsible for the production of those reacting substances. (2) In the second kind of test, the suspected organism is isolated in pure culture (Fig. 81) from the infected area (throat, feces, blood, etc.), and its identity is proved by its agglutination or precipitation with known antisera—antisera obtained from another known to have that particular disease.

Each of these two kinds of tests is obviously just the reverse of the other. In the first kind as described above, the serum of the patient is the unknown factor and the laboratory organism is the known factor. In the second kind, the serum is the known factor and the organism of the patient is the unknown. These two types of tests may be more briefly contrasted as

- (1) Patient's or unknown serum against known organism.
- (2) Patient's or unknown organism against known serum.

Both kinds of tests may be made in a given disease, as in typhoid. The blood is examined to see if typhoid agglutinins are present. Blood is collected from the ear lobe, finger or any convenient place, in capillary tubes and allowed to stand until the serum has separated; or, more simply, a few drops of blood may be dried upon a clean glass slide, though this cruder method is questioned by many. The blood or serum thus obtained is diluted with physiological salt solution, and typhoid bacteria are added to see if agglutination occurs. If the result is positive (the typhoid bacteria being agglutinated), no other test is necessary. Should the result be negative, the second kind of test should also be tried. In that test a small amount of the fecal discharge is diluted, and varying amounts spread over agar plates to allow the typhoid bacteria to develop (Fig. 81). Their detection is often aided by adding to the agar such a dye as "brilliant green," which inhibits most types of intestinal bacteria, while allowing typhoid to develop. The colonies developing on such agar plates are studied, and those having the characteristic typhoid appearance are selected for testing against known agglutinins; bacteria taken from these colonies are mixed with an antiserum known to contain

agglutinins for typhoid bacteria. If the bacteria thus obtained are agglutinated it is thereby demonstrated that the individual is harboring typhoid bacteria. If, in any given case, negative results are obtained by this test also, one or both tests must be repeated as long as any doubt exists as to the cause of the disease, more conclusive evidence ordinarily being obtained as the disease progresses.

Negative Results.—Negative results here as elsewhere, are never conclusive. The blood will not give a positive test for agglutinins until a certain borderline of agglutinin production has been reached. A negative result may mean only that the individual has not yet formed enough agglutinins to show up in such a test. Since people vary greatly in their degree of resistance to invading organisms, an individual may continue to give negative results day after day—and even throughout the attack. It is not difficult to understand that even in serious illness the agglutinin tests sometimes give only negative results; the agglutinins formed are insufficient to protect the individual or to show up in such laboratory tests.

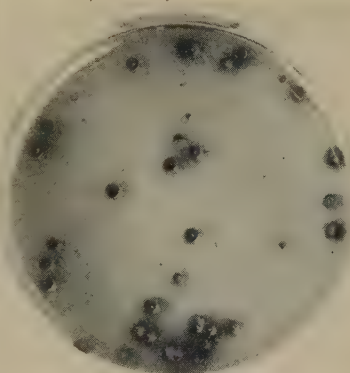


FIG. 82.—This plate, made from the same fecal sample as the preceding, shows only *Bacterium coli*, because such a weak dilution was made that only the more numerous *Bact. coli* were represented in the amount used. (Endo's medium). Photograph by Heins from plate made by the Bureau of Laboratories, Health department of the City of New York.

The tests depending upon the isolation of the causal organism have their special difficulties also. Other organisms present in the material examined (sputum, feces, etc.) may outgrow the ones searched for. The causal organism may not be present in the very small amount necessarily used in making the agar plates (Fig. 82). Sometimes, as in typhoid, where the organisms develop in glands in the lining layers of the intestine, and are irregularly ejected into the intestinal cavity, they may not show up in the fecal material even though samples are taken on several

successive days, and examined by the most careful and elaborate technique.

Other Difficulties of Technique.—As mentioned earlier, group agglutinations may mislead the investigator unless the dilutions are properly made (p. 111). Slides and other materials should be carefully prepared or cared for, as slight amounts of acids, alkalies, and salts may mislead the worker by inhibiting agglutination or causing a false agglutination. Besides, laboratory or stock cultures of bacteria often lose their agglutinating power, or vary markedly in the dilution showing agglutination and should be checked by known antiserums at intervals; for example, one strain fell suddenly from 8,000 to 4,000 in the dilution capable of causing agglutination.

Occasionally non-agglutinable strains of organisms are obtained even in diseases where agglutination is characteristic; *e.g.*, non-agglutinating strains of typhoid have been isolated from the spleen, gall-bladder, etc. This resistance on the part of the organism probably has a definite relation to its invasiveness or virulence, making it less easily affected by the protective substances in the body as well as under laboratory conditions.

Normal Agglutinins.—Another difficulty to be considered in such tests lies in the fact that “normal agglutinins” may occur, as shown earlier for antibodies in general (See p. 62). The newborn child often possesses such normal agglutinins, probably due to substances transferred with the mother’s blood, as they tend to disappear later. Adults may also possess “normal agglutinins,” probably explainable by the well-known fact that even in health, bacteria may invade the tissues (*e.g.*, the intestinal environs, or the portal circulation) and so, probably, excite the production of a small amount of agglutinin or other antibodies before the invading organisms are wholly overcome and destroyed. (See p. 62.)

All the foregoing difficulties make it necessary that such tests as these agglutinin and precipitin tests should be made by responsible and experienced people, carefully checked or controlled wherever possible. It is also essential that the result of such tests should be considered in combination with the physical or clinical evidence.

Diseases Diagnosed by Agglutination Methods.—Among

the diseases involving agglutination are such intestinal infections as cholera, typhoid, paratyphoid and dysentery. Of these, the typhoid test is the most common in our country, and the test oftenest used is the first one described on page 118, the direct examination of the blood for agglutinins—commonly known as the Widal test. Often, however, in typhoid, and very commonly in the other intestinal infections named above, the second kind of test is used, in spite of the difficulty often incurred (p. 119) in isolating the specific causal organism. This second test may succeed when the other fails because agglutinins do not always appear early in the diseases; they naturally follow rather than precede the development and multiplication of the invading organisms. In typhoid, for example, the agglutinins rarely appear before the end of the first week and often not till well toward the end of the second week. On the other hand, the typhoid bacillus is isolated from the blood oftener early in the disease than in the later stages. Attempts to obtain the organism in culture are often unsuccessful by the end of the second week or even earlier.

Agglutinin Tests for Respiratory Diseases.—Three diseases which affect the respiratory areas—pneumonia, meningitis, and glanders—may be diagnosed by agglutination. In pneumonia, sputum or material from the naso-pharynx is usually examined for pneumococcus organisms, and the type determined by agglutination so as to aid in selecting the type of antiserum to be given the patient. (See also p. 80 and p. 122.)

Meningitis carriers are picked out by isolating from the nasal cavities meningitis bacteria, which agglutinate when mixed with known antimeningitis serum. In actual cases of meningitis, serum is drawn from the spinal canal to determine the presence

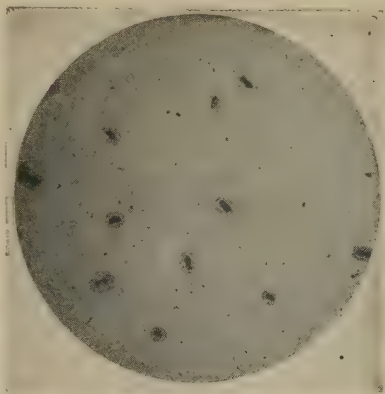


FIG. 83.—Pneumococcus organisms, stained by Huntoon's method to show the capsules. HUNTOON, *Journal of Bacteriology*. Photograph by Dunn.

of meningitis bacteria, which are positively identified by finding that they are agglutinated by antimeningitis serum. Corroborative tests (cloudiness, white corpuscles count, sugar, albumin) are also made on the spinal fluid; the spinal fluid and the blood are apparently not rich enough in agglutinins to use them in tests against known meningococcus organisms.

In pneumococcus infections, as described elsewhere (p. 80) it is important to determine early the type of pneumococcus. This may be done in several ways: (1) One method depends upon isolating the organism from the sputum, lung exudate, etc., and testing small amounts of the pure cultures thus obtained against the different types of antisera—antisera made by different horses against the respective types of pneumococcus. Rapid methods of isolation, using special media such as rabbit's blood, have been developed to utilize this method.

(2) A second method takes advantage of the fact that such exudates as the sputum contain substances that can be precipitated when brought into contact with pneumococcus antiserum. In other words, instead of using the bacteria in agglutination tests as described in the preceding paragraph, we use liquid obtained from the areas affected by the bacteria. The sputum is treated (heated in normal salt solution, alkaline hypochlorite solution, etc.) and a clear fluid is finally obtained. This fluid is added to the respective types of antiserum, precipitation occurring only in the tube containing the related antiserum. This may be indicated by a general cloudiness or precipitation or as a definite precipitated area or "contact ring" where the added fluid comes in contact with the serum, (much as in Fig. 79).

(3) The third method of diagnosis is called the mouse inoculation method. Sputum, containing the pneumococcus organisms, is injected into a white mouse (peritoneum) where the organisms multiply for 5 to 8 hours. The exudate then obtained from the peritoneal cavity is specially prepared (centrifuged, etc.), after which the sediment and the supernatant fluid are tested against the different pneumococcus antisera in various ways; the clear fluid being used to show precipitation with the different types of serum as described in the preceding paragraph, or the bacterial sediment may be used with the sera to show agglutination.

The glanders test is chiefly made by the first of the above

methods. To the diluted serum of the horse to be tested dead glanders organisms are added. Resulting precipitation of bacteria (Fig. 79), and final clearing of the supernatant fluid in 72 hours or less is taken as a positive result. Although glanders is usually an infection of horses, the disease may occur in other domestic animals and also in human beings.

As stated earlier, *Proteus vulgaris*, (*B. proteus*), an organism apparently not at all related to typhus fever, may be used to diagnose typhus fever. Since the causal organism of typhus fever has not yet been definitely determined this is a fortunate coincidence, and the *Proteus* organism (strain 19), in dilutions of 1 to 50 or over, is at present satisfactorily used with the patient's serum in testing for typhus fever.

Whooping cough is not diagnosed by agglutination tests, although the pertussis bacterium can be readily agglutinated by rabbit antiserum. This is due to the fact that the blood of human cases is not rich in agglutinins. The opposite form of the test—the patient's organisms tested against antisera known to be rich in agglutinins, such as the rabbit antiserum—is not generally used either, because the pertussis bacterium is only with difficulty distinguishing from other bacilli common in sputum. (See lysin test for whooping cough.)

Agglutination Tests in Other Diseases.—Agglutination tests are used for certain diseases in addition to those just described. In Malta fever, the patient's serum is tested against cultures of the causal organism, *Bacterium melitensis* (*Micrococcus melitensis*); agglutination when the patient's serum is diluted 1000 or more times indicates Malta fever.

In tuberculosis, agglutination tests have not been found of any real value; the dilutions used are much lower than with any other infection—but one to 10 or 15. In gonococcus infections agglutination tests are not used for diagnosis, though the various strains of gonococcus may be differentiated by such tests in much the same way as we type the pneumococcus strains. We are, however, just attacking the immense task of typing the various disease organisms.

Standardization of Antisera.—While the abundance of agglutinins in a given antiserum is indication of good body reaction, the amount of agglutinin bears no definite or constant

relationship to other anti-substances, and measurement of the agglutinins present is not an exact measurement of the whole or total protective value of a serum. However, in diseases in which agglutinins are characteristically developed, a good antiserum is rich in agglutinins; and in treating such diseases, it would therefore be sensible to discard any antiserum not coming up to a given agglutinin titer or strength. Commercial typhoid, men-



FIG. 84.—Precipitation tests for human blood. Tubes 1 to 10 (top row) showing positive reactions, contain human blood, each specimen dried for one month on various material (1 silk handkerchief; 2, tweed cloth; 3; black dress fabric; 4, dark green cloth; 5, coarse green cloth; 6, coarse red cloth; 7, kid glove; 8, blanket; 9, very coarse material; 10, flannel). The controls, tubes 11 to 20, with negative reactions contain the same series of blood samples, but have yielded negative results because they were tested against anti-ox serum instead of anti-human serum. The slight cloudiness of the lower tubes is not true precipitation. NUTTALL, Immunity and Blood Relationships, Macmillan and Cambridge University press.

ingitis, and dysentery antisera may, therefore, be described in terms of agglutination strength, especially dysentery antisera (See p. 138).

Agglutinins Against Other Types of Cells.

—Bacteria are not the only cells which can stimulate the production of agglutinins and precipitins. Practically any foreign cells

(*e.g.*, white corpuscles, red corpuscles, gland cells) can cause the production of such antibodies when injected into other kinds of animals. Indeed the production of such antibodies may be taken to indicate that the cells inducing it are foreign. To illustrate: a guinea pig will not form antibodies if injected with any of his own tissues (except possibly the eye lens protein), nor, normally, if injected with red blood cells or other tissues of other guinea pigs. But antibodies are formed by the guinea pig following the injection of red blood cells or other tissues of other kinds

of animals such as man, horse and sheep. In fact, the amount of reaction of injected animals gives a basis for judging the degree of relationship between the injected animal and the animal providing the blood for injection.

Relationship in zoölogical families (horse, dog, rabbit) is supported by such agglutinin tests. These tests support the acknowledged relationship of the horse and the ass, the fox and the dog, the sheep and the goat and the domestic and the wild pig. In monkey groups interesting support has been found with regard to the degree of relationship between the various groups of monkeys: orang-outangs, chimpanzees and gorillas; and man has been shown by agglutinating blood tests to be more closely related to one group, the chimpanzees, than some of the monkey groups are to each other.

Agglutinin Tests for Blood Transfusion.—Human blood differs somewhat in different individuals and four distinct types have been observed, when tested by the tendency to form agglutinins against other specimens of human blood. One of these four groups is very large, containing nearly 50 per cent. of all human beings. It is fortunate that so many people fall into one group, as emergencies sometimes occur where it is not possible to test blood before using it for transfusion. Agglutinin tests are made by adding red corpuscles from the volunteer to a sample of the serum of the patient. If the red corpuscles added are agglutinated or precipitated it shows that the patient's serum treats them as foreign; and it would be dangerous to transfuse the blood of that volunteer into the patient, for it is only when tests show such agglutination that the red corpuscles are destroyed by the white corpuscles of the patient. Transfusion of such blood would be dangerous because it would mean caring for a lot of disintegrating foreign protein (See p. 193); and it would not, of course, accomplish the usual aim in transfusion—the addition of active oxygen-carrying red corpuscles. (See also red corpuscle lysins and blood transfusion.)

Blood Tests as Legal Aids.—The reaction of one animal to the red blood cells of another kind of animal is usually so specific that the serum of small laboratory animals (rabbit, guinea pig) may be used to identify the kind of blood with which they have earlier been injected or sensitized. If the blood cells are intact

this identification can be made by means of a hemolysis test. (See p. 156.) Often, however, the only blood in question is in a blood stain; but even so, a definite conclusion can be reached by means of a precipitation test. The broken-down blood washed out of the stain with salt solution is floated over a given immune serum in a test tube; a definite ring at the point of contact (much as in Fig. 79) identifies the blood cells in the stain as the kind used to produce the immune serum.

By this means, in such situations as a murder trial, a blood stain can be shown to have been caused by human blood, and not, as the defendant claims, by the blood of some other animal, as for example, a horse. Fresh serum from an animal sensitized against human red cells properly diluted is put into a test tube and to it are added some of the red blood cells washed out of the stain. If they are precipitated they are human cells, and the stain in question was, of course, made by human blood. Whether or not the stain is horse blood, may be shown by the same kind of a test, substituting, of course, for this part of the test, the serum of an animal sensitized to horse red blood cells.

These blood cells reactions are so reliable that they may be used to determine the kind of meat present in such preparations as sausage and "chopped beef," though a modified method is necessary with meats that have been heated. Even dried blood soaked from boards and chopping blocks has been successfully used in this way as legal evidence. The age of the blood stains affects these tests less than one would suppose. In one case a blood stain sixty-six years old gave satisfactory results when so tested; so did embalmed or mummified blood forty years old. Similar claims have been made for very old mummy blood—for one sample two thousand years old, and for another five thousand years old.

Precipitins Against Unformed Proteins.—Such proteins as milk, serum and white of egg are called unformed or non-cellular proteins. Sensitized animals may also be used to identify these substances; the precipitin reactions of these animals are so delicate that their serums may be used to determine whether a given substance is the white of a hen's egg or a duck's egg; whether it is human milk or cow's milk. The possibility of the application of such tests to food adulteration is at once evident.

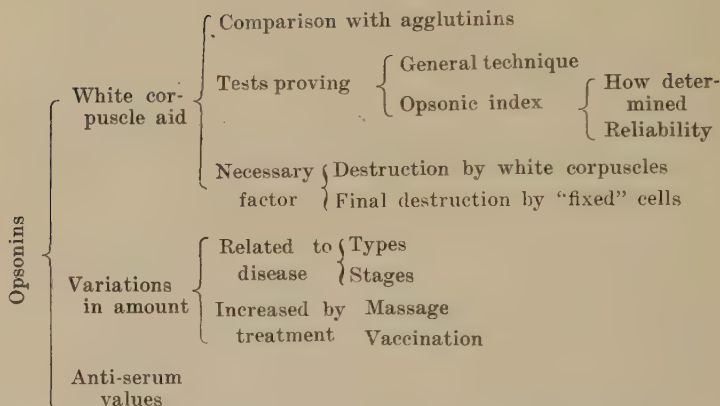
These reactions are so similar to the phenomena discussed under Anaphylaxis that the reader is here referred to Chapter X, where human sensitiveness to such food proteins as milk and eggs, and to such foreign cells as horse skin cells and cat hairs, and pollen (hay fever) is briefly discussed.

STUDY SUGGESTIONS

1. In what ways may agglutinins help the body in overcoming bacterial infections?
2. Describe a test for disease in which the patient may be shown to have specific agglutinins present in his blood.
3. Describe a disease test in which the organisms obtained from the patient's blood, mouth or nose exudate or intestinal discharges are identified by showing that they are agglutinated by a given or known antiserum.
4. What diseases are satisfactorily tested for by agglutinins or precipitin tests?
5. Show how agglutinins or precipitins may be used to identify blood in meats, blood stains, etc.
6. How could such adulteration as horse meat for beef be detected?
7. How could you determine whether hen eggs or duck eggs were being used in a given egg powder, icing preparation, etc.?
8. Tests showing agglutination of red blood cells give results in 15 to 30 minutes; how does this compare with the time interval in tests involving the presence of lysins which dissolve the red corpuscles (see Chapter VIII)?
9. How can we use the blood content of mosquito stomachs to determine the animals mosquitoes feed upon? Of what use might such knowledge be in controlling a disease spread by an insect feeding upon other hosts besides man, as in malaria or in sleeping sickness?

CHAPTER VI

OPSONINS (Tropins)



OPSONINS described on page 49 as aiding the white corpuscles in the destruction of foreign organisms, are so named (from the Greek *opsonium*, a relish) because of the greater avidity or relish with which white corpuscles take in and digest bacteria, when these substances are present. At least as early as 1895, Metchnikoff noticed that the serum of immune animals aided white corpuscles in the destruction of bacteria, the number of bacteria ingested and destroyed being greater than when normal blood or serum was used with the white corpuscles (Fig. 85). *Tropins* and the less definite term *stimulins*, are other names for such substances. (See p. 129.)

Agglutinins and Opsonins.—Like agglutinins, opsonins can not single handed bring about destruction of bacteria; each of these antisubstances acts as an aid to the white corpuscles in this work. Neither opsonins nor agglutinins have any definite quantitative relation to any other antibodies, such as the bactericidal substances or lysins which may be present in an immune serum (Fig. 86). Opsonins, like agglutinins, are specific for the organ-

isms which excite or stimulate their production, anti-staphylococcus opsonins aiding in staphylococcus destruction and anti-tuberculosis opsonins acting in tuberculosis (Fig. 94).

Although normal blood may be shown to contain varying amounts of normal opsonins, these normal opsonins are, however, unlike the specific opsonins formed during disease or after vac-

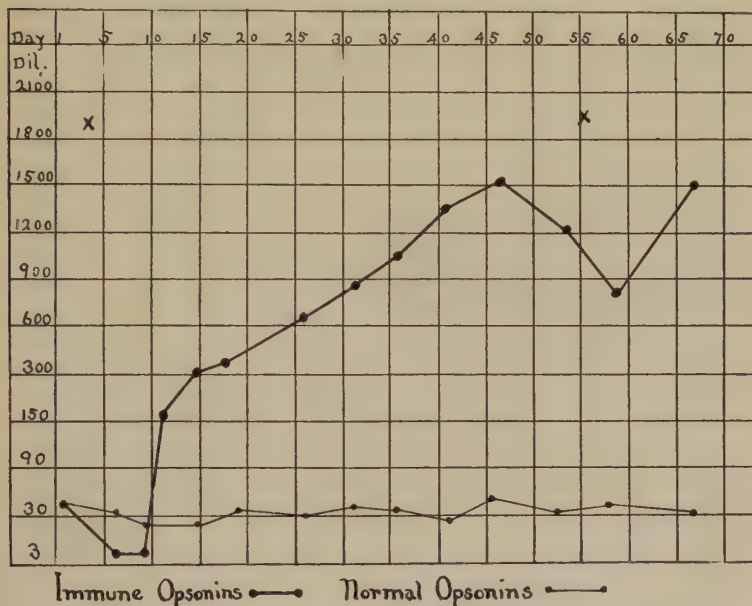


FIG. 85.—A chart showing the great amount of immune opsonins in blood when compared with normal opsonins of a second or control animal; X indicates the injection of killed tuberculosis organisms in the first animal. MEAKINS, *Journal of Experimental Medicine*.

nation. The normal opsonins are not only less abundant, but they differ also in being less resistant to heat than the opsonins of immune blood. Workers who emphasize the difference between normal and immune opsonins designate the latter as tropins. In this respect opsonins differ from agglutinins and lysins, for both normal agglutinins and lysins apparently differ from immune agglutinins and opsonins mainly in amount, not in kind.

Tests for Opsonins.—Meningitis and streptococcus anti-serums have a marked opsonic effect and opsonins may be the most valuable antistances present. Opsonins also play an important

part in the body reactions against staphylococcus and tuberculosis infections, and aid substantially in combating pneumococcus and gonococcus infections.

It might be expected therefore; that in a given disease or infection, a measurement of opsonins present would give some indication as to how vigorously the body is reacting (See p. 135). The

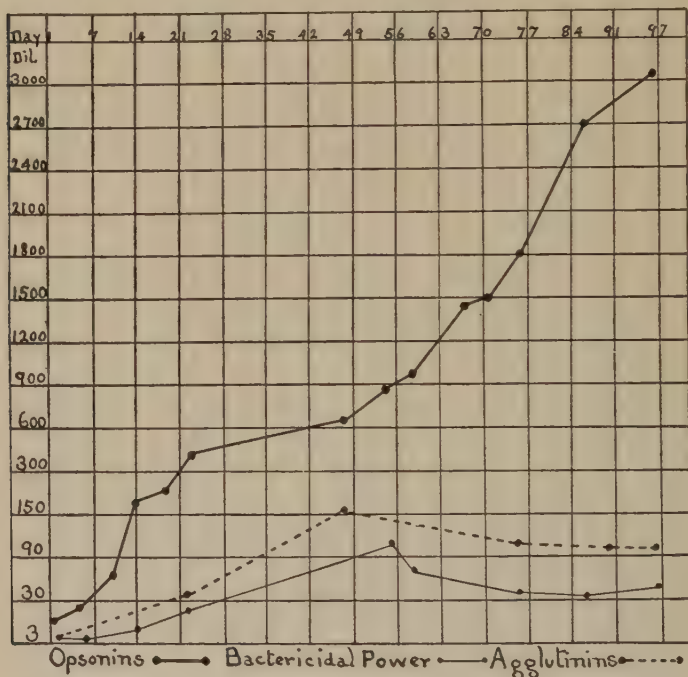


FIG. 86.—This chart shows that the various antibodies do not increase proportionally against a given organism; tests were made for each of the three antibodies shown every 5 to 9 days for 97 days. MEAKINS, *Journal of Experimental Medicine*.

presence and relative abundance of opsonins in a given blood or serum, is demonstrated by showing how the addition of such blood or serum affects the white corpuscle destruction of bacteria.

While this determination is far from simple, it is not really difficult to understand, either in theory or as a matter of laboratory practice. To determine how much aid such opsonins are giving to a tubercular patient, for example, we add a little of his

serum to a mixture of white corpuscles and tuberculosis organisms. The serum is obtained by taking a small amount of blood from the patient (*e.g.*, ear or finger prick); after it has clotted, a little of the serum is drawn off into a specially marked or graduated capillary pipette (Fig. 89). Similar carefully measured amounts of tuberculosis organisms and of washed white corpuscles from a non-tubular source (a normal human or such laboratory animals as a guinea pig or rabbit) are measured off, and all three are well mixed together to insure the uniform suspension of the opsonins, bacteria and white corpuscles. (The corpuscles are washed to make certain we are dealing with only the patient's opsonins—not opsonins from the blood furnishing the corpuscles for the test.) This mixture is allowed to stand (fifteen minutes) at body temperature, thus providing favorable conditions for white corpuscle action. A little of this triple mixture is then spread upon a glass slide, stained, and examined under a microscope to determine the extent of the white corpuscle ingestion of bacteria. This may be measured in two ways: (1) by counting the total number of bacteria ingested by the first hundred corpuscles seen, or (2) by estimating what per cent. of the first hundred white corpuscles seen have ingested bacteria. Results are more easily estimated by the second of these two methods, as it may be “impossible to count the bacteria ingested by even one cell for the leucocytes simply gorge themselves.”

The Opsonic Index.—Since normal serum has some opsonic power, exactly how much special opsonins are going to help a patient combat a disease, such as tuberculosis, could be determined only by comparing the results obtained with the patient's

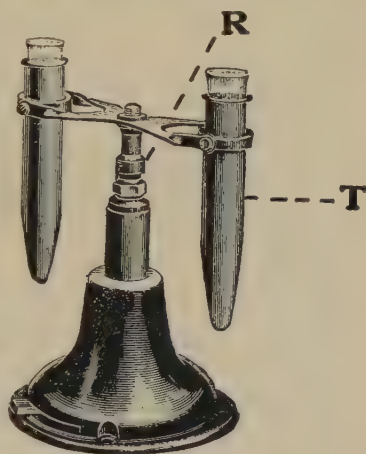


FIG. 87.—A centrifuge, with a revolving shaft, R, which spins rapidly and whirls the tubes into a horizontal position, the heavier part of the contents (such as corpuscles in blood) being thrown outward (centrifugal force) to the bottom of the tube.

serum and with normal serum. A control test is, therefore, made by repeating exactly the same procedure described in the preceding

paragraph, substituting a non-tubercular normal serum—often the worker's own serum—in the place of the patient's serum. Wright, who did much to develop the technique of opsonin measurement, used a mixture of five normal serums for this control part of the test, thus securing a less variable normal serum for the control.

Any difference in the white corpuscle activity is attributed to the difference between the normal serum and the patient's serum. If, for example, in the normal serum mixture, the first hundred white corpuscles counted showed ten white corpuscles containing bacteria, and the tubercular serum yielded a count of twenty-one containing bacteria, the relative power of tubercular serum to the normal serum would be as twenty-one to ten, or $\frac{21}{10}$. These relative values may be expressed as 2.1, and would technically be spoken of as an opsonic index of 2.1, the interpretation being that the patient's serum is more than twice as efficient as the normal serum in destroying the invading organisms. Results are given in such "index" terms. To quote, for example, from hospital reports, the opsonic index of one patient's serum against staphylococcus infection causing a series of boils was but 0.62, much less than normal. Another patient who had been vaccinated with staphylococcus organisms to increase his resistance against a similar condition showed an opsonic index of 2.4 (Fig. 90).

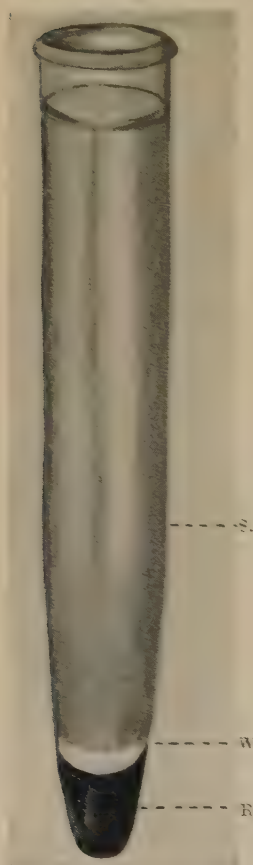


FIG. 88.—Tube of blood, after centrifuging, to separate the white corpuscles for making opsonic tests. S, serum; W. C, white corpuscles; R. C, red corpuscles. After THOMAS and IVY. Applied Immunology, J. B. Lippincott Co.

Difficulties in Determining the Opsonic Index.—The technique employed demands very careful work. The amounts

measured in the capillary pipettes are very small, and slight differences in bulk might mean a big difference in the relative number of bacteria and white corpuscles. Any variation of this kind would mean a similar difference in the opportunities or



FIG. 89.—An opsonic pipette, with a marked tip by which are measured the bacterial suspension, white corpuscles, and the patient's serum which are drawn in separately, a little air separating them as indicated. These three substances are then mixed and kept at body temperature (15 to 30 min.), when slides are made, stained, and ingestion of bacteria noted through the microscope.

chances for bacterial ingestion, and would yield unreliable results. Recently important differences have been shown to be due to such matters of technique as slight variations in the acidity of the liquid used in washing the white corpuscles or making up the final suspension. Most workers feel that about

OPSONIC INDEX DETERMINATION

Bacteria + Patient's serum + White corpuscles	Bacteria + Normal (control) serum + White corpuscles
<hr style="width: 100px; margin: 0 auto;"/> 46	<hr style="width: 100px; margin: 0 auto;"/> 20
← Percentage corpuscles ingesting bacteria →	
$\frac{46}{20} = 2.3$ Patient's Opsonic Index	

FIG. 90.—Method of determining a patient's opsonic index.

10 per cent. error is to be expected. It may be several times 10 per cent., and it is claimed that even the results of experts may not agree; in one instance, working on the same case, one expert showed that the patient had an opsonic index lower than normal, while another estimated the opsonic index to be higher than normal. There is, however, a great difference in the chances of error

in the several special methods for determining the opsonic index, and the method to be used should be carefully considered. (See also p. 138.) Besides these mechanical difficulties, the patient's physical condition (fatigue; loss of blood, as in hemorrhage) affects the results obtained.

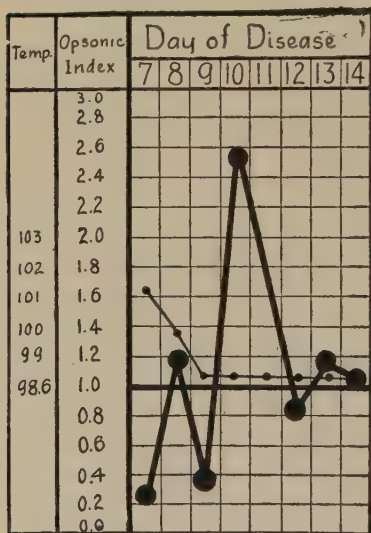


FIG. 91.—The heavy line indicates the opsonic activity of a patient against *Streptococcus pyogenes* on the 7th to 14th day of an attack of erysipelas. This curve shows quite typically the early period with the opsonic index below normal (0.2 to 0.7), the rise in this index (average 2.0, ranging from 1.4 to 4.4) with a falling temperature and improvement, and the abrupt fall to normal in 1 to 3 days. (The light line is the temperature record.) TUNNICLIFF, *Journal of Infectious Diseases*.

Opsonins as Aids in Prescription.—Other mechanical difficulties affect the value of opsonic tests in treating a patient. The time involved in making such counts is also an important consideration affecting the adoption of opsonic tests in determining the progress of disease. The preparation, separation, washing and proper dilution of the white corpuscles is itself no small task. Several hours are not too much to allow for all the details involved in comparing the patient's opsonic activity with that of a normal individual. This means that in medical practice, the observation of clinical or physical symptoms and the determination of the opsonic index can not be really contemporary. Only under the best hospital

or sanitarium conditions can the clinical and opsonic reports be less than a few hours apart, and often they are twenty-four, or even several days apart. This means that an opsonic index is, therefore, often valuable only in corroborating the physician's diagnosis or treatment rather than in aiding him in making his decisions or prescribing treatment.

One of the most useful applications of the opsonin tests is in conditions such as joint infections, where there are no open lesions to aid in diagnosis. An example very widely quoted in

this connection was the case of a patient having a swollen wrist joint. (Fig. 93.) To determine whether the inflammation was due to gonorrhoea or to tuberculosis the opsonic index test was taken both for gonococcus and for tuberculosis organisms, under varying conditions; throughout, the tuberculosis index remained about normal, 0.94 to 1.1, while the gonococcus index ranged from 1.3 to 2.4, thus indicating that the inflammation was due to gonorrhea and not to tuberculosis.

Aside from this diagnostic application of the opsonic tests little practical value attaches to their use in the actual treatment of disease, and while variations in the opsonic index are used in special investigations, they are not used as a routine guide for the administration of treatment.

Relation of Opsonins to Final Bacterial Destruction.—

Quite recently it has been claimed that the fixed body cells are more important in bacterial destruction than the white corpuscles—that the white corpuscles hold the bacteria but temporarily, their ultimate destruction occurring mainly in the spleen and lymphatic areas. If this is so, the rate at which opsonins aid the white corpuscles in ingesting bacteria, would be an important initial factor, but would give no accurate measure of the actual rate of bacterial destruction, and an opsonic index could not be an exact index of the patient's resistance.

Variations in Opsonic Index Related to Types of Disease.—A low index is often associated with a definitely localized infection, as a gland or a joint, or with a quiescent focus. The explanation of this is that in such instances relatively small

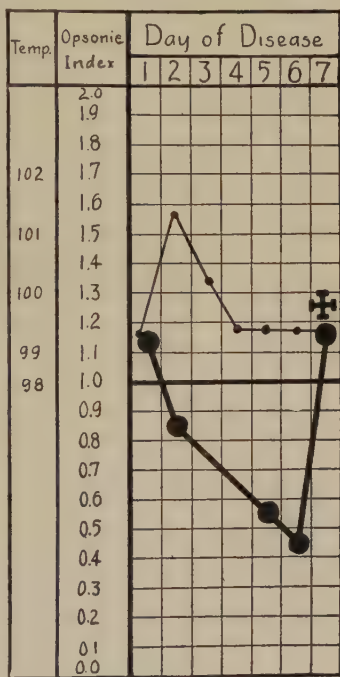


FIG. 92.—Here the opsonic curve is measured against *Streptococcus pyogenes* in a fatal case of erysipelas. Note the contrast to the curve of recovery in the preceding figure. The light line represents the temperature changes during the same interval. TUNN:CLIFF, *Journal of Infectious Diseases*.

amounts of irritating substances are distributed from the focus, and so, little body reaction (opsonins) results. A low index may also be found in more or less chronic or at least long continued infections, such as acne or boils, where the opsonins are often considerably below normal (.4 to .8).

A high index is often associated with active lesions, especially

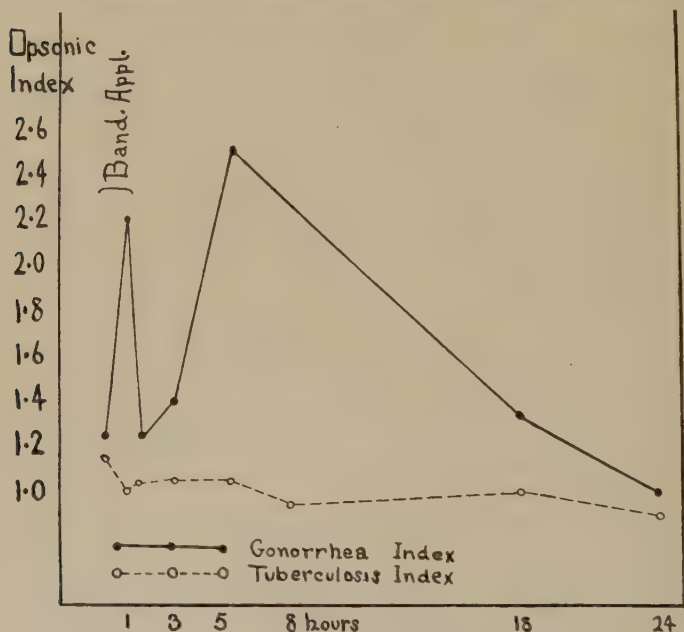


FIG. 93.—An increased opsonic index obtained by bandaging the infected area. The tuberculosis index remained the same, while the gonorrheal index varied, as shown, thus indentifying the infection as gonorrheal. Redrawn from Wright, *Studies in Immunisation*. Constable.

in pulmonary tuberculosis, and probably indicates that the body is being forced to do its utmost to overcome the irritating organisms.

Increasing the Opsonic Reaction.—In cases with a low index, the body may be stimulated to increased reaction by an increase in or by greater distribution of the irritating factors. In more or less chronic cases (boils, acne, tuberculosis) this may be done in two ways: First, by massage or pressure of the localized

infection (such as tubercular joint) thus distributing the irritating organisms and exciting more general and greater body reaction (Fig. 93). This method may, of course, be actually dangerous, distributing too much irritating material, thus overreaching the mark, injuring the body tissues and failing to produce a compensatory increase in opsonins or other antisubstances.

The second method of increasing the opsonic reaction is by repeated injections of killed bacteria or vaccines. In staphylococcus infections, such opsonin increase may be readily demonstrated. To illustrate, a patient suffering from "chronic" boils was found to have an opsonin index for staphylococcus (Fig 95) considerably below normal—about .6. He was vaccinated with a few billion killed staphylococcus organisms, and (after the usual brief drop) his opsonic index became established at about 1.3. A few days later he was again vaccinated, reaching an opsonic index of about 2.0 which was followed by complete recovery. (See p. 132.)

Zinsser, summing up the results and conclusions of several workers in this field says that, "in many of the infections of man the resistance of the patient is roughly proportional to the opsonic index—and that properly spaced inoculations with suitable quantities of dead bacteria (vaccines) will raise the opsonic index and lead to the recovery in many of the localized subacute and chronic conditions."

Other Tests Based on Opsonins.—Besides the more or less successful method of estimating an individual's body reactions to disease by measuring the opsonic value of his serum, opsonins are capable of another important laboratory application. The value of curative antisera—especially meningitis antiserum—may be measured in terms of opsonin values. While the United

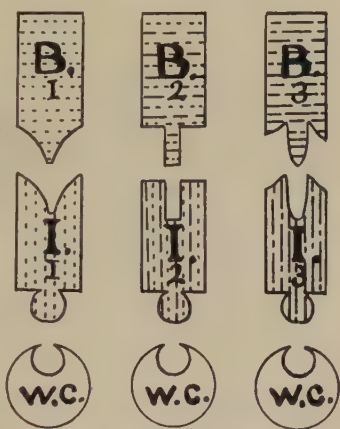


FIG. 94.—Diagrammatic representation of the formation of specific opsonins adapted to different bacteria, but alike in their power of working with the white corpuscles, as indicated by the similarity of the immune body-corpuscle juncture.

States Public Health Service does not use the opsonic reactions of any serum as an official standard, the opsonic value may be used as an aid in deciding whether a given serum should be passed. (See p. 124.) For example, a given anti-meningitis serum, which is questionable according to the agglutinin or complement-fixation tests, may be passed on its opsonic rating. Opsonin values are quoted more often for anti-meningitis serum than for any other serums. Investigators have reported the presence of opsonins in a 1:5,000 dilution of meningitis serum;

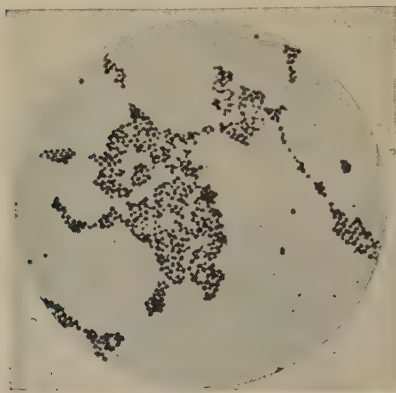


FIG. 95.—*Staphylococcus aureus*, a common pus organism. Williams, Brown and Earle.

this is apparently high, for reliable workers make such statements as a "commercial serum having tropins for the two main groups of meningococci in a dilution of 1:4,000 is considered excellent."

Besides the difficulties already mentioned (p. 132) which are experienced in securing accurate and consistent results in measuring opsonins, another objection to using opsonins as a basis for the standardization of

serums has been the fact that opsonin values do not correspond to the protection values of a given serum. Recently Evans with improved (Neufeldt) technique, has shown for each of a series of antisera, a surprising correspondence between the opsonic strength and the protective value. While the protective value of a given serum does not lie in its opsonic value only, it is possible, however, that the measurement of antiserum strength may be much more definitely established by these newer methods.

STUDY SUGGESTIONS

1. What are opsonins?
2. What three substances are used in testing for opsonins? Why should a control test be made?
3. Show how opsonins may aid in diagnosis of one disease.

4. Give the difficulties which may affect the accuracy of determining a patient's opsonic index.
5. Explain why variations in a patient's condition in a given infection (tuberculosis, boils) might be expected to correspond to changes in his opsonic index.
6. Find in a text book on bacteriology or immunology a series of opsonin tests made on one individual, and chart the reactions obtained as in Fig. 85.
7. List from an advanced text in bacteriology the human diseases in which opsonins are thought to be important body aids.

CHAPTER VII

WHITE CORPUSCLES

White Corpuscles	Phagocytic activity	Primitive rather than specialized function
		Dependence on opsonins
		Relative activity of types of white corpuscles
	Increase in number—Phases and types of disease	
	Other variations in activity	Health and disease
		Age
		Different types of animals
White Corpuscles	Extracts	
	Bacterial resistance	Capsule relations
		"Virulence"
		Aggressins
	Tests	Number related to specific diseases
		Progress during disease

As early as 1870, bacteria were seen in white corpuscles, but strange as it seems to us now, their presence in the corpuscles was thought to indicate that the bacteria were extending their destructive influence to the blood cells. Later, the theory of the protective activity of the white corpuscles was advanced, in a speculative way, but it was not until 1883 that Metchnikoff first showed experimentally, that white corpuscles both ingest and digest bacteria (Fig. 96). He and his associates also showed that this activity was greater in the presence of immune serum. (See Agglutinins and Opsonins.)

Phagocytic Action a Digestive Process.—It is not uncommon to find that the phagocytic action of white corpuscles is viewed by the layman as a very unusual type of cell activity, indicative of a high degree of specialization in the white corpuscles. Phagocytosis is, however, essentially the same process that occurs constantly in one-celled animals, protozoa, as they engulf and digest food particles.

This type of cell activity is not only characteristic of small protozoa, such as the ameba (Fig. 97), but may be demonstrated in favorable tissues in many higher animals as well (Fig. 98). As illustrations of such tissues the following may be mentioned: (1) The lining cells of the intestines of certain blood-sucking worms or leeches which engulf and digest red blood cells; (2) the intestinal lining cells of mollusks which similarly ingest food particles; (3) various gland cells in the human body—lymph

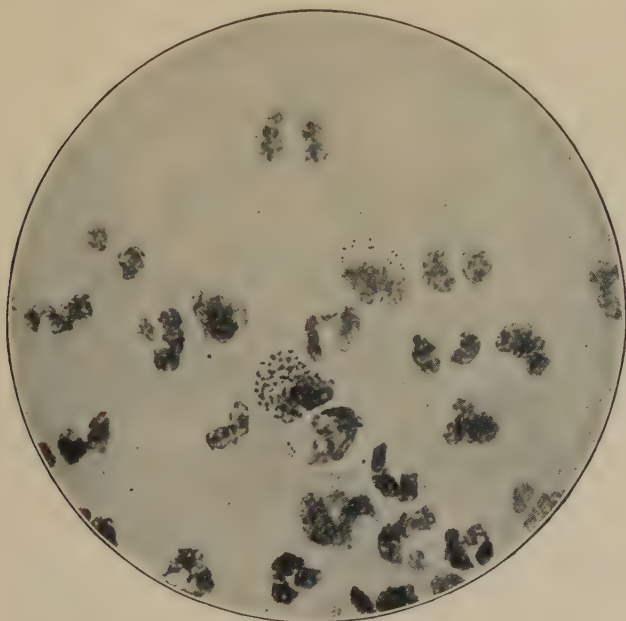


FIG. 96.—Numerous polynuclear white corpuscles from pus; two near the centre have ingested numerous gonococcus bacteria, (*Neisseria gonorrhoeae*). ADAMI and McCRAE, Textbook of Pathology. Lea and Febiger.

nodes, liver and spleen—which are constantly causing the destruction of red blood cells; (4) the alveolar cells of the human lung which surround and destroy foreign particles, including bacteria brought to them in the inspired air; and (5) the bacterial destruction due to the lining cells of the blood vessels and the abdominal cavity.

From these illustrations, therefore, we may conclude (1)

that phagocytic activity of the white corpuscles is far from peculiar and may be interpreted as a retention of a very primitive characteristic rather than the development of a highly specialized power; and (2) that phagocytosis is not limited to the motile white corpuscles, but is a function of many fixed cells.

Phagocytosis by Fixed Cells.—The importance of certain fixed tissue cells in bacterial removal has been shown by several investigators in various animal experiments, and includes the

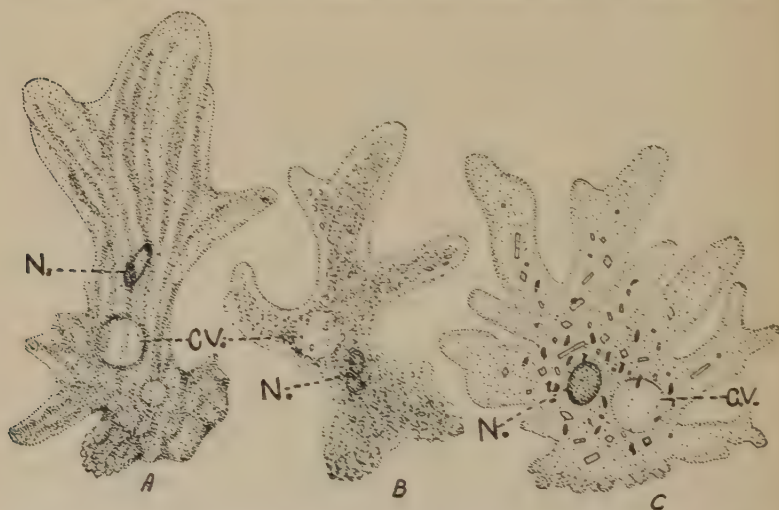


FIG. 97.—Three different amoebas showing the irregular body or cell outlines; changes in these projections allow movement and the engulging of food particles. (N, nucleus; c v., contractile vacuole.) SCHAFER, *Amoeboid Movement*, Princeton University Press.

activity of the liver in pneumococcus infections in guinea pigs, the spleen and liver in typhoid infections in rabbits, and the spleen, liver and lungs in streptococcus infections in rabbits. Such activity on the part of the fixed tissues may lessen the importance attaching to the opsonic index, and some writers have claimed for these fixed cells greater results than those achieved by the white corpuscles themselves.

Against this last opinion must be balanced the fact that in the tissues, such as the spleen, where the white corpuscles are temporary agents, they are none the less important ones; for whether the white corpuscles are the final destructive forces, or

mainly temporary agents transferring their ingested bacteria to the spleen and the lymphatics for final destruction, they are necessary to the phagocytic process. (See p. 145.)

The Factors Involved in Phagocytosis.—It will probably be best to discuss phagocytosis under three headings: (1) The part played by serums, (2) the rôle of the white corpuscles, and (3) the bacteria exciting or stimulating this white corpuscle activity.

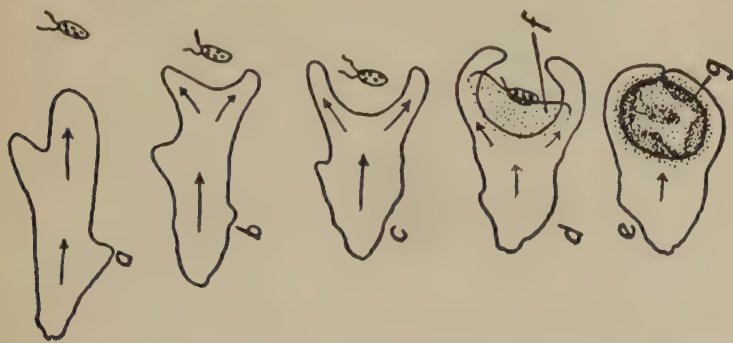


FIG. 98.—Diagrammatic representation of the stages (a-e) in the movement of an amoeba, and the ingestion of food. SCHAFER, *Amoeboid Movement*, Princeton University Press.

Serum Factors in Phagocytosis.—While investigators disagree as to the rate at which white corpuscles alone can ingest bacteria, they all agree that such ingestion is much more rapid when immune serum substances, agglutinins and opsonins, are present.

The importance of the preliminary action of agglutinins as an aid to phagocytosis has been discussed in the chapter on Agglutinins and Precipitins. Opsonins, in the chapter preceding this, have been shown to be necessary not only for the ingestion but also for the complete digestion of any considerable number of bacteria; without such opsonins the ingested bacteria may retain life and sometimes full virulence. (See also p. 145.)

Nevertheless, the great importance of these serum substances should not blind us to the fact that though the white corpuscles can not complete these destructive processes unaided, they are themselves nevertheless indispensable to the process. How indispensable, may be seen from the fact that bacteria such as

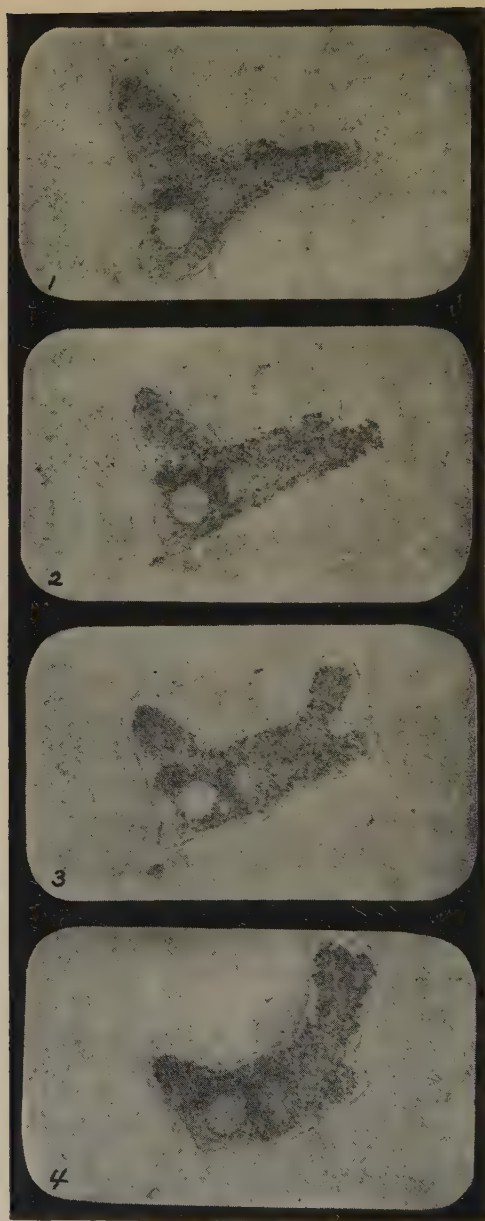


FIG. 99.—Three units, from a moving picture film, showing the same amoeba in movement at intervals of 15 seconds. The photographs are placed to show movement toward the right. RICKER, U. S. Public Health Service.

pneumococci (*Diplococcus pneumoniae*) may multiply rapidly in the most potent immune serum after the mechanical destruction of the white corpuscles. Similarly, pneumococci multiply in defibrinated human blood or serum because few or no phagocytes are present after defibrination. Anthrax apparently offers another illustration of the importance of white corpuscles with relation to opsonins, for while dogs are very resistant to anthrax, the organisms multiply rapidly in normal dog serum.

White Corpuscles and Phagocytosis.

—It is sufficient for our purpose to consider only the two main types of white corpuscles: (1) The polynuclear leucocytes, which have two or more nuclei, marked ameboid activity (Fig. 99) and ingestive power and which are produced mainly in the bone marrow, and (2) the lymphocytes, with less ameboid and ingestive power, having but a single nucleus, and produced mainly in the lymph nodes.

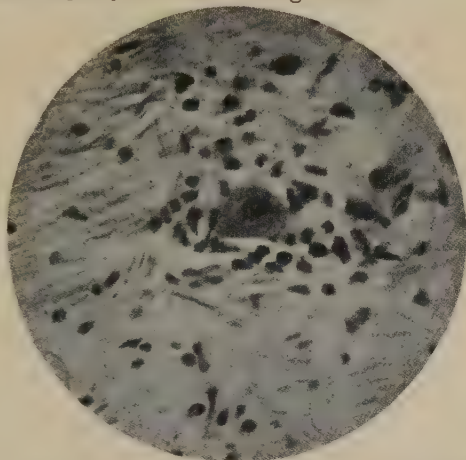


FIG. 100.—Nerve tissue showing the invasion of phagocytes which are surrounding the ganglion cells undergoing disintegration.

NEAL, *International Clinics*, J. B. Lippincott Co.

Most of the ingestion and destruction of bacteria is done by the polynuclear leucocytes. With regard to the rôle of the lymphocytes, investigators are less in agreement. It is probably safe to attribute to the lymphocytes the following activities: (1) They accumulate in localized or early infections apparently forming an important initial stage of the white corpuscle reaction; (2) they are important in the final disintegration of broken down cells and foreign substances; (3) they may help in chronic infections or in infections due to very resistant bacteria,

may also be an increase in the number of white corpuscles. This has been attributed to the irritating substances resulting from the destruction of the tissue cells. Such responses are less marked than the responses made to bacterial stimulation.

White Corpuscle Changes in Specific Diseases.—While the white corpuscle count may, as described, show variations during the course of a given attack or infection, it may vary also with the kind of disease. This is readily understandable as the relative importance of the other reactions of the body, such as

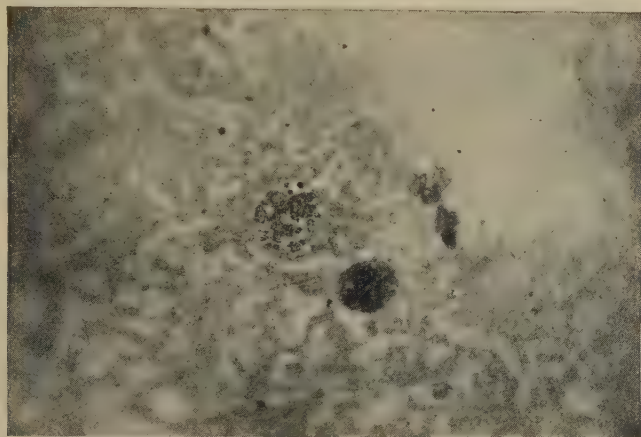


FIG. 103.—A blood smear showing phagocytosis; the large white corpuscle in the centre has ingested or swallowed pneumonia organisms. (Photograph by Bull.) BROADHURST, Home and Community Hygiene, J. B. Lippincott Co.

lysins or antitoxins, would affect the need for extra corpuscle activity. Normally the white corpuscle count is about 5,000 to 9,000 per cubic millimetre. Increase in the number of white corpuscles is characteristic in pneumonia, while a decrease is more often found in influenza. (See also p. 151.)

White Corpuscles as Carriers of Infection.—White corpuscles have, by virtue of their ameboid activity, the power of migrating through the capillary walls and into the tissues. This is doubtless often an advantage in the rapid movement of these phagocytes to the infected focus, but it is conceivable that it may increase the number of local infections, as white corpuscles are not always able to digest or destroy all the bacteria

they have taken in. In such a situation resistant bacteria may be transported to a new area, and undeterred by the white corpuscle that conveyed them, set up a new focus of infection. Such transfer is not easily demonstrated, as the surface allowing the passage is not always inflamed. The location of infections, otherwise difficult to account for, as in osteomyelitis and tuberculosis, are sometimes attributed to such white corpuscle transfer. This method, of course, is the exception rather than the rule, and should not detract from our conception of the white corpuscles as important protective agents.

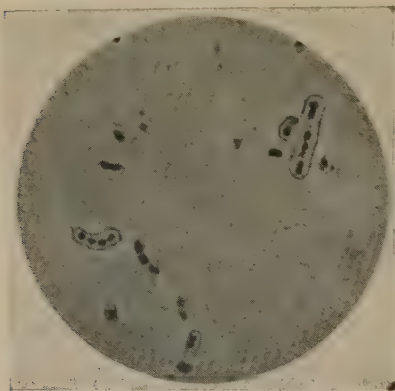


FIG. 104.—*Streptococcus mucosus*, stained to show the capsule so characteristic of this species. (Photograph by Dunn.) HENSTON, *Journal of Bacteriology*.

Variations in Normal Phagocytic Activity.—

White corpuscles vary not only in their relative abundance, but in the vigor of their activity. Their phagocytic power varies in health and disease in a given individual. Age differences have also been noted, the activity of the white corpuscles of new-born children being less than that of adults. Corpuscles from normal individuals differ somewhat in their power to dispose of such bacteria as the staphylococcus and tuberculosis organisms.

In the lower animals, there is great difference in the relative activity of the normal white corpuscles: the white corpuscles of the frog, for example, aiding greatly in destroying injected anthrax bacteria, while the corpuscles of guinea pigs and rabbits show very little power of this kind.

White Corpuscle Extracts.—Much of the white corpuscle activity is explained on the basis of the enzymes they contain. Different protein-splitting enzymes have been studied, these enzymes being held accountable for such activities as dissolving of fibrin (clotted blood) or the disintegration of bacteria.

Experimental work has been done following this general

line, in an effort to demonstrate that similar results may be obtained by white corpuscle extract (obtained by subjecting washed white corpuscles to distilled water). Experimental animals and infected human beings have been treated with such white corpuscle extracts, and beneficial effects claimed in infections due to streptococcus, staphylococcus, pneumonia, influenza and meningitis organisms. Horse blood may be used for preparing such white corpuscle extracts, and a rather large dosage may be given, as much as 100 c.c. every four to six hours. This treatment is not yet practically established, and its value, at present, seems greatest in localized infections.

Relation of Bacteria to Phagocytosis.—The phagocytic powers of the white corpuscles are not exercised against all kinds of bacteria; diphtheria bacteria, for example, are little affected by this activity. Phagocytosis may occur with some members of a group of organisms, and not with others, *e.g.*, a capsulated species of streptococci resists the phagocytes, while the uncapsulated types are readily engulfed. This same difference is seen within a given species of bacteria, where there is a difference in individuals in capsule formation; white corpuscles have been seen to select uncapsulated anthrax bacteria from masses of capsulated ones. Often the formation or presence of a capsule (Fig. 104) parallels the possession of greater virulence or pathogenicity, thus supporting the view that the capsule is an "earmark of virulence." According to Zinsser, "Habitually capsulated bacteria, like the Friedlander bacillus and *Streptococcus mucosus*, are of fairly consistent virulence, while in other microorganisms like the pneumococci, anthrax bacillus, plague bacillus, and certain other streptococci, the formation of a capsule goes hand in hand with an increase of virulence."

The waxy capsule of tuberculosis organisms may help account for the fact that their destruction is not so great as their rapid ingestion by the white corpuscles would lead us to expect. This resistance, it is claimed, may be due to the lipoids prominent in the capsule.

In other cases definite bacterial resistance exists without the presence of capsules; in explaining these cases refuge is usually taken in the vague term "virulence" which covers not only this resistant quality but also actual invasive properties.

Another way of explaining the resistance of bacteria attributes to them the power to form special substances—enzyme-like substances—which neutralize or destroy the reacting substances of the body, or paralyze the polynuclear white corpuscles, and so give the bacteria a relative immunity to the white

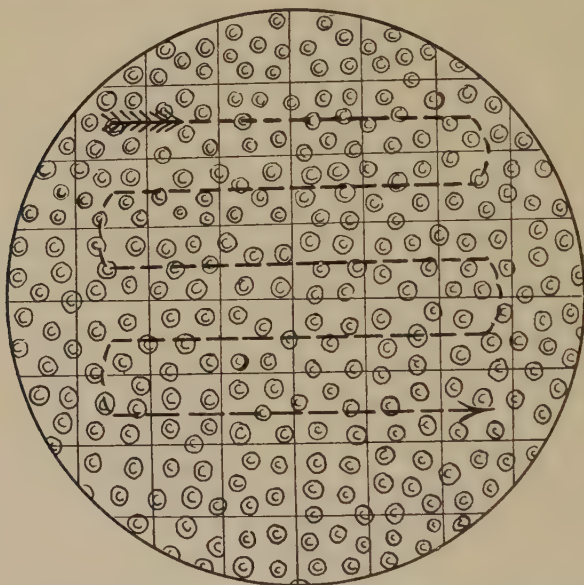


FIG. 105.—Part of a specially marked blood-counting slide showing the red corpuscles. Moving the slide as indicated by the arrow makes it possible to count all the organisms in a representative number of the marked spaces, without repetition. There are slight variations in the technique used (dilutions etc.) for estimating red corpuscles, white corpuscles, and bacterial vaccines, but the general principle is the same. After DA COSTA.

corpuscles, lysins, etc., and aid the bacteria in their invasion of the tissues. These special substances are called aggressins (See p. 56); and belief in their existence has been supported by experiments showing increased virulence in experimental infections, if sterile material from an abscess is inoculated in addition to the related type of bacteria. To these aggressins have been attributed also other powers, such as the paralysis of the phagocytic white corpuscles.

Tests Based on White Corpuscle Counts.—In spite of the

fact that white corpuscles and opsonins are interdependent, we have not yet demonstrated any definite parallel or ratio in these two responses to infection. Indeed, there may exist definite contrasts in these two responses in a given case, such as a low opsonic index with a high white corpuscle count in a staphylococcus infection. It may well be that our present methods of determining such values are not delicate and accurate enough to show the real relationship that exists—or that these differences may be due to a more or less temporary overproduction in one reaction in the effort to compensate for the delayed manifestation of the other reaction.

However that may be, white corpuscle counts and their variations can be used as laboratory aids much more readily than opsonin determinations. This may be due to the greater ease with which accurate white corpuscle counts are determined.

The diagnosis of disease is sometimes materially aided by making blood counts, thus securing evidence of the marked reduction of overproduction of the white corpuscles already mentioned on page 147. Reduction (leucopenia), for example, is characteristically found in uncomplicated typhoid, and in influenza and, often, in tuberculosis; a marked increase in the white corpuscles (leucocytosis) is equally characteristic of pneumonia, streptococcus and staphylococcus infections, especially in deep-seated abscesses. As characteristic comparison illustrating these differences we might cite the following counts from reports on individual cases in recent medical journals: Leucopenia: typhoid, 2,000; measles, 3,600; influenza, 2,800; tuberculosis, 1,000. For leucocytosis: erysipelas, 20,000; cerebral meningitis, 34,000 and 47,000; severe

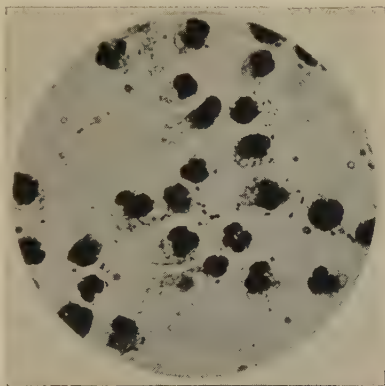


FIG. 106.—Spinal fluid from a case of meningitis, showing white corpuscles and pneumococcus bacteria. (x 700). SCHOENING, Photograph by Sands.

diphtheria, 30,000, and pneumonia, 56,000, 115,000, 151,000 and 245,000.

There are not, however, many human diseases in which such blood counts prove practical aids; and, as might be expected, such indications are lacking in the diseases, such as diphtheria, in which phagocytosis plays little or no part. Death often occurs even when the white corpuscle count indicates a high degree of reaction against the invading bacteria. Too much importance must not be attached to the count alone, for as Emerson well states it, a high count simply means that the patient is putting up a vigorous fight.

Variations in the number of the white corpuscles are not, on the whole, so helpful in making the diagnosis of the disease itself, as in determining an individual's progress during an attack of a given disease or in ascertaining his response to prolonged vaccine treatment and, thereby, the most favorable dosage.

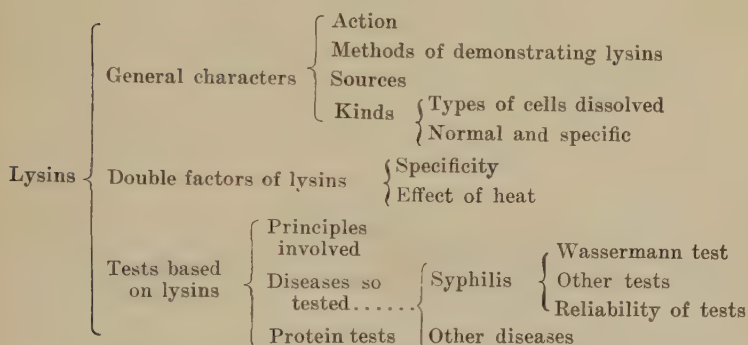
STUDY SUGGESTIONS

1. Show that the phagocytic power of white corpuscles resembles the activity of many other kinds of cells.
2. What three factors enter into phagocytosis?
3. Describe the probable relationship of agglutinins and opsonins to phagocytosis.
4. Consult a text book on pathological bacteriology or diagnosis and calculate for one or more diseases the per cent. increase recorded for the patient's white corpuscle count.
5. Some twenty years ago a doctor recommended that small abscesses be treated by local injections of distilled water; what possible connection is there between this recommendation and the belief that the phagocytic action of white corpuscles is due to the enzymes they contain?
6. What reasons are advanced to explain the variation in the susceptibility of different bacteria to phagocytosis?

CHAPTER VIII

LYSINS

(Including Complement Fixation Tests).



IN 1886, Nuttall showed that normal blood contains special substances, lysins, which destroy bacteria. When bacteria are acted upon by lysins they become granular and swollen, and are finally completely dissolved. This result must not be confused with the destructive action of white corpuscles, for, as was later shown, this type of dissolution takes place without the presence of white corpuscles, that is, by the action of the serum alone. Later work showed, also, that these lysins are much greater in immune serum (Fig. 107) than in normal serum, most normal serum having, in comparison, very little destructive power. In one of Nuttall's early experiments he demonstrated that one cubic centimetre of rabbit's blood contained enough lysin to destroy over 50,000 anthrax bacteria, causing their complete disappearance in four minutes.

Methods of Showing Lysin Action.—The presence of lysins may be demonstrated by making slides at intervals from a mixture of bacteria and immune serum and comparing the number of organisms visible with the aid of a microscope. In another method the serum and bacteria are mixed in larger amounts in a test tube, and at intervals, small but definite

amounts are taken out and used in making agar plates to determine how many live bacteria remain. After these plates have been incubated to allow the live bacteria left in each amount to develop into colonies, the colonies are counted, the difference in

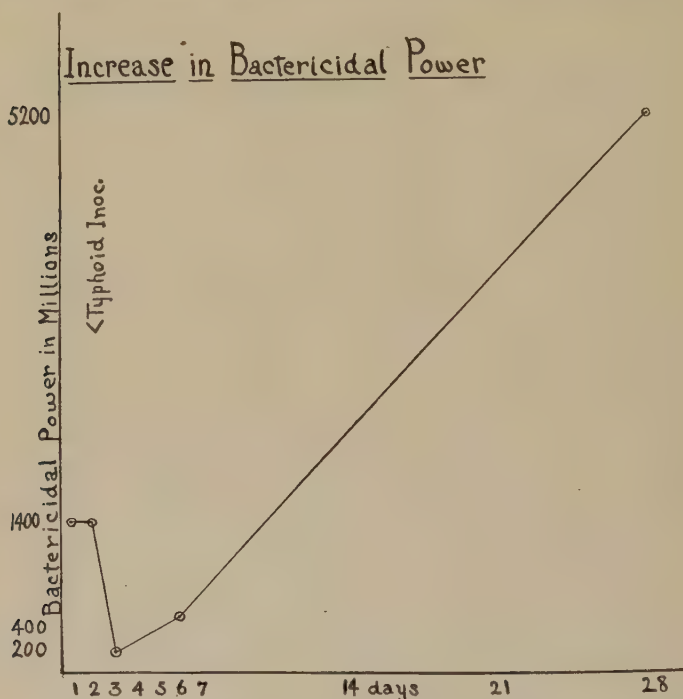


FIG. 107.—On the second day, the subject was inoculated with typhoid vaccine. After the initial drop, the bactericidal power reached normal in about a week, and continued to rise during the three-week observation period that followed the inoculation, Redrawn from WRIGHT. *British Medical Journal*.

the counts of the various agar plates made including the rate at which the bacteria have been killed off.

Source of the Lysins.—The production or action of lysins has no relation to the presence of opsonins or white corpuscles. As indicated in the opening paragraph, this dissolving power of the serum is not to be confused with the similar destructive action of white corpuscles. The end results are the same, but the

process is very different—so different that the claim that the serum lysins are formed in the white corpuscles cannot be accepted. One of the most striking differences is that the action of the serum lysins can be destroyed by heating the immune serum (60° C.) and then renewed by adding a little normal blood, although normal blood itself lacks this destructive power; white corpuscles or their extracts cannot be reactivated in this way.

White corpuscles are therefore evidently not the source of the lysins. Some investigators have, on the basis of their experi-

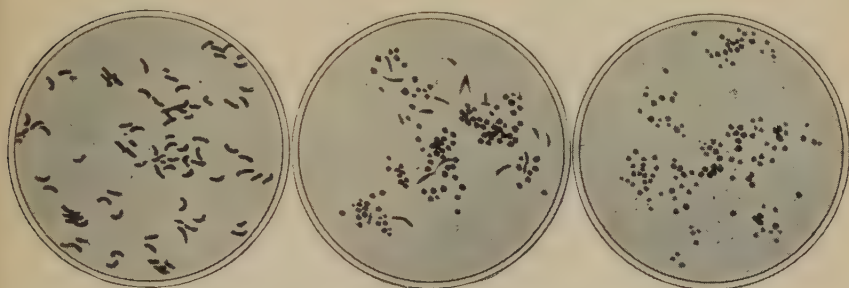


FIG. 108.—Stages in the lysis or dissolution of cholera organisms (left to right) before final dissolution occurs. KARSNER and ECKER, *Principles of Immunology*, J. B. Lippincott Co.

ments, designated the liver or the thyroid as the lysin producer, but as yet we can not positively attribute to any special gland or tissue the production of lysins. (See p. 48.)

Red Corpuscle Lysins.—Lysins are formed not only against bacteria but against other foreign cells. Most of the experimental and laboratory work with other than bacterial cells, has been done with red blood corpuscles. If the red corpuscles of one animal such as the cow, are injected into an animal of another species, the guinea pig, for example, the injected animal develops lysins to aid in disposing of the “foreign” red cells injected. Whether or not such lysins have been formed, may be demonstrated (Plate 3.) by mixing together in a test tube the serum of the injected animal (guinea pig in the above discus-

sion) and washed red corpuscles of the same species as those with which the animal has previously been injected (cow).

If no lysins have been formed, the red blood corpuscles in the mixture finally settle by gravity to the bottom of the tube, leaving a colorless liquid above them. If lysins are present, they dissolve out the red coloring matter, and the colorless remnants of the corpuscles settle to the bottom of the tube, but the hemoglobin being now in solution, does not settle with the corpuscles, and the liquid in the tube does not clear but remains red throughout. Lysins that affect red corpuscles and their contained hemoglobins in this way, are called hemolysins in contradistinction to those affecting bacteria (bacteriolysins).

Specific Character of Lysins.—Lysins are specific, whether formed against bacteria, or against other types of cells, such as red corpuscles. For example, lysins formed by a guinea pig against the red blood cells of a cow do not hemolyze human red cells, and vice versa. It is therefore possible to test the blood serum of such injected animals against samples of blood (obtained from meat, blood stains, etc.), and so determine the kind of animal from which the meat or blood originated.

To illustrate, a man accused of murder may be cleared or convicted by a study of the blood stains that led to the charge, and which he claims were caused by an animal other than man. In such a test, the blood corpuscles are washed out of the stain, and then they are tested against the serum of a laboratory animal (*e.g.*, rabbit) which has been injected at suitable intervals with human red corpuscles. If the corpuscles washed out of the stain are human corpuscles, the serum of such a rabbit will dissolve them and the test tube contents will remain red (Pl. 3.). If the corpuscles from the stain are not human, they will not be dissolved by the serum and will settle unbroken to the bottom of the tube, and the contents will gradually clear (See Plate III). The results are evident in one to three hours; (an additional period of 12 to 24 hours at ice box temperature often aids in interpretation).

It is, of course, also easy to prove or disprove the defendant's statement regarding the kind of blood (cow, pig, dog, etc.) in the stain under investigation, by using in a similar way, the serum

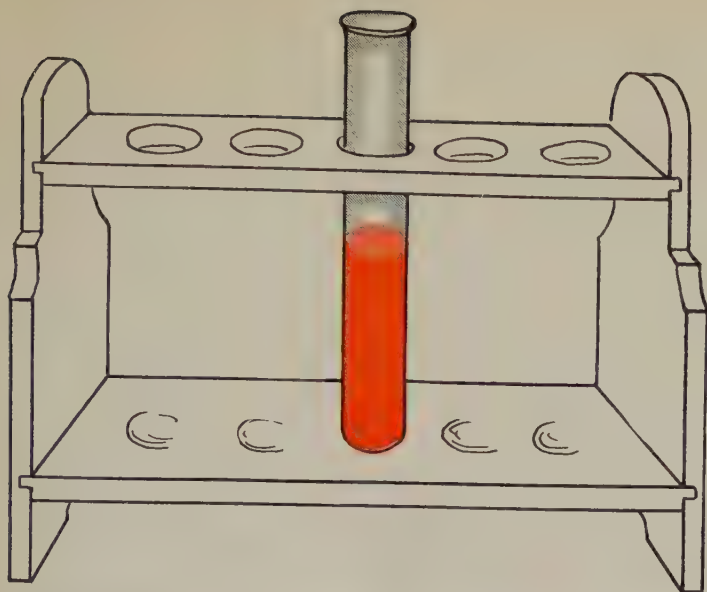


PLATE III A.—Tube showing dissolving (lysis) of red corpuscles; the red coloring matter has escaped from the corpuscles, and such tubes will therefore not clear on standing.

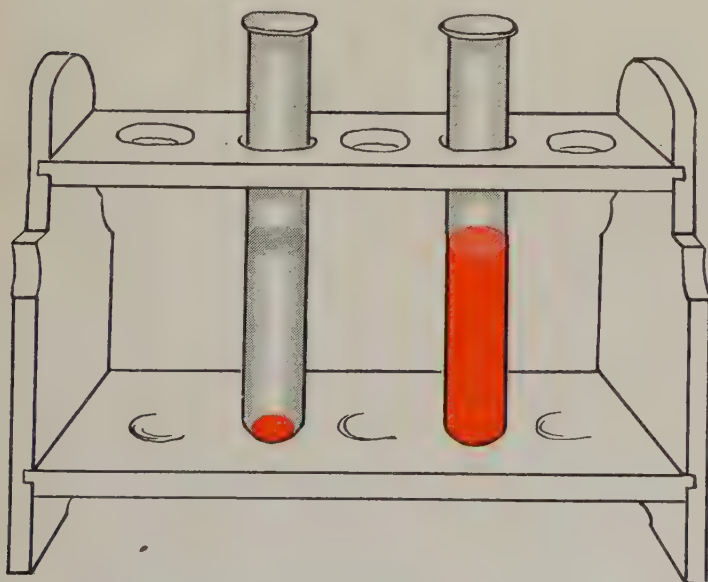


PLATE III B.—Human blood stains give a positive test (right tube) with the serum of a laboratory animal which has been injected with human red cells and negative results (left tube) with the serum of a laboratory animal injected with red cells of a dog, pig, etc.

of a laboratory animal which has been injected with the corpuscles of the animal the defendant mentioned.

Blood tests of this kind—both in murder trials and in other legal situations, such as determining the type of meat sold—are more commonly used in European countries than in the United States. In this country more dependence is placed on delicate chemical tests (substances present, types of crystallization) as in the hemin test. (See also blood tests under agglutinins and precipitins.)

Long-dried blood stains do not lend themselves so well to this test, as the red cells may be already broken up. In such cases the precipitin test (p. 126) would be employed to ascertain the kind of blood in the stain.

Normal Lysins for Human Red Corpuscles.—The blood of one person may contain lysins against the red corpuscles of another individual. If time allows, tests are made before transfusing blood from one person to another, in order to make sure that the blood to be transfused is not too “foreign” to yield the expected benefits. (See also p. 125.) In cases of anemia or where much blood has been lost, as in severe wounds or hemorrhage, no benefit would result from transfusing blood corpuscles that are straightway destroyed, for if the transfused corpuscles are to serve as “carriers of oxygen” they must remain intact in the blood stream of the patient. Whenever possible, therefore, the blood offered for transfusion, is first tested against the patient’s serum to see if the serum contains any lysins against the volunteer’s red corpuscles. Usually samples are taken from several volunteers, and tested at the same time, to avoid unnecessary delay in finding suitable blood for transfusion. The blood selected for transfusion, will, of course, be that in which the corpuscles were so like the patient’s that no lysis takes place, the whole or unbroken corpuscles settling to the bottom of the tube and the liquid becoming clear. (See also p. 125.)

Bacterial lysins, because of their specific character, lend themselves to diagnosis of disease, and tests based on their presence are now used in several diseases (See p. 159), the patient’s serum being tested against the suspected organism to see if special lysins for those organisms are present. Unlike the case with red corpuscles, such mixtures of serum and bacteria give no color or

other visual evidence regarding serum lysins. The microscopic and plate count methods described on page 153 were developed for indicating and measuring bacterial lysins. At present, however, a peculiarly complicated but interesting method of demonstrating their presence or absence has been devised (to be described later, p. 160), which is based on one of Bordet's contributions to the subject of lysins, *i.e.*, that a lysin is not a single but a double substance or factor.

Lysins not a Single Factor.—A puzzling inconsistency between the results obtained with immune serum in living animals and in test tube experiments, led to the discovery that a lysin is made up of two substances: one substance common to normal as well as immune blood, and the other a special immune substance specific for the organism which called it into existence.

In early guinea pig experiments with cholera, it was found that the immune serum formed by a guinea pig against cholera, contained a lysin for cholera organisms. This lysin killed cholera organisms equally well, whether used in test tube experiments or injected with virulent cholera organisms into a guinea pig. If the immune serum were heated to 60° C., however, it lost its power to act in test tube mixtures with cholera organisms, but strange to say, still protected a guinea pig as effectively as the unheated immune serum.

This inconsistency was explained by Bordet when he showed that a lysin contains two substances. One substance is common to normal as well as immune serum and is very susceptible to heat, being destroyed at 60° C. or even lower temperatures. The second substance is the immune part of the lysin; this is usually found only in immune serum, and is quite resistant to heat, surviving 60° C.

Both of these substances are necessary for the lysis or dissolution of bacteria. In the test tube mixture of cholera and serum, therefore, no action takes place if heated serum is used. Since heating the serum, however, destroyed only a substance with which the normal guinea pig is well supplied, but left the immune part of the serum, the heated serum was therefore as effective in protecting a guinea pig, as the unheated serum.

But if, to the test tube containing the bacteria and heated immune serum mixture, a little normal guinea pig serum is added, the action will now be exactly as effective as if *unheated* immune

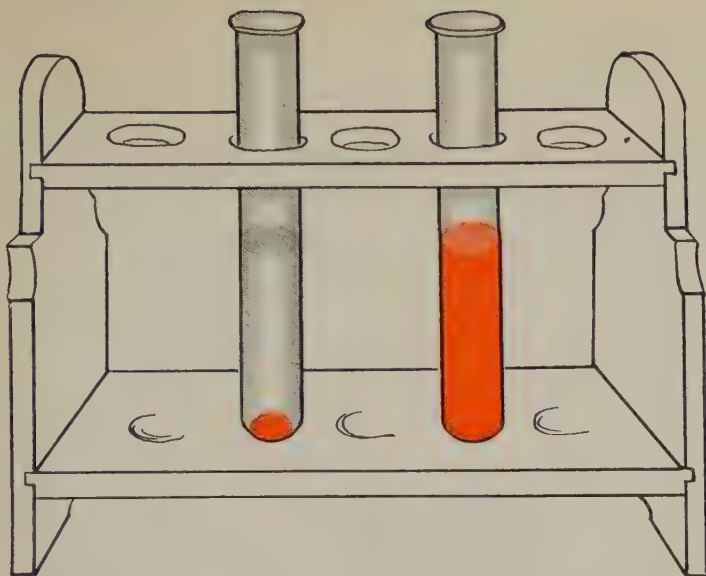


PLATE IV A.—Results using the heated and the unheated serum of laboratory animal (guinea pig) injected with red blood cells of another animal (rabbit). The unheated serum (right tube) contains both the immune bodies and complement, therefore can dissolve rabbit red blood cells; the heated serum (left tube) has had its complement destroyed and cannot complete the combination (P. 158) necessary to dissolve the red blood cells, and they have settled to the bottom unbroken.

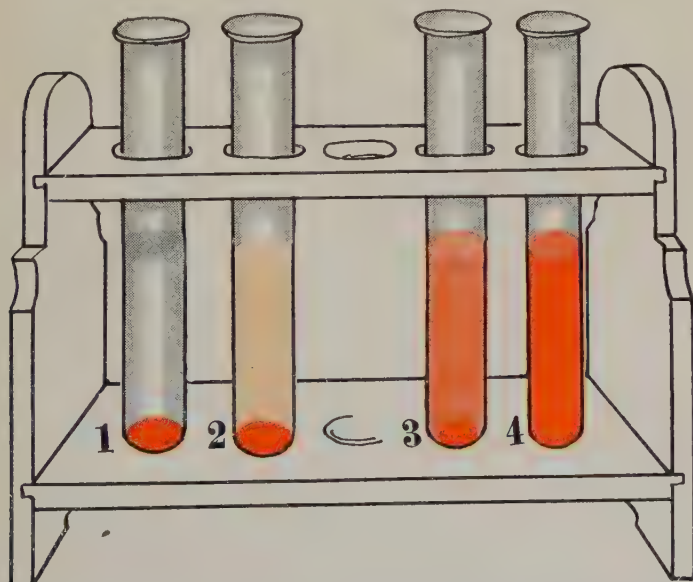


PLATE IV B.—In a complement-fixation test, such as the Wasserman test, a completely positive result is shown in 1, a wholly negative one in 4; grades between the two are shown in 2 and 3. Patients improving under treatment progress from 1 to 4, intermediate grades being recognized (+++, ++, + &c. of laboratory reports).

serum had been used, showing that the normal serum has completed or complemented the action of the heated serum. The constituent supplied by normal serum is therefore termed *complement*.

Complement not Specific.—As stated above the complement factor is not specific, but is found in normal blood. The same complement aids in complementing the action of the immune substances formed in the blood of such widely differing animals as man and the guinea pig; and normal guinea pig complement is commonly used to complete the action of human serum in many well established tests. And the kinds of cells which are destroyed by the aid of the complement include the greatest possible range, for normal complement from the same animal can be used satisfactorily in tests against bacteria (pertussis, in whooping cough tests, or gonococci, in gonorrhea); against protozoa (the spirochætes of syphilis); and against red blood corpuscles (of man, cow, sheep, rabbit, etc., in blood tests).

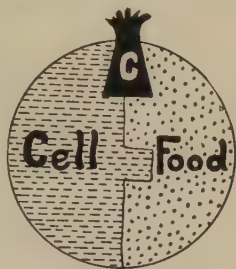


FIG. 109.—Wholly diagrammatic representation of the source of complement present in normal blood. Ordinary "metabolic" processes such as cell utilization of food probably account for the presence of complement in normal blood.

The preceding examples are given in an attempt to illustrate how a single type of complement can bind together many very different kinds of immune bodies and invading organisms. (Complement itself is probably a double substance, but since we are here interested only in the general phase of the subject, it is not possible to consider the lesser differences that do exist in various complements). Complement is apparently not increased during disease, after vaccination, etc. While the source of complement has not been demonstrated, it may, as some workers believe, be formed in the white corpuscles. It is quite possible that complement plays a very important rôle in the normal body, *e.g.*, in the utilization of foods (Fig. 109). At any rate, its importance in the destruction of foreign cells is as great as if it were as specific as the other antibodies, for the immune part of the lysins cannot work without complement.

Diagnósis of Disease.—Blood determination tests by means of lysins have already been described (p. 157). Lysins may also

be used to prove that an individual has or has had certain diseases by showing that his serum contains special or specific lysins against the disease in question, just as described on page 155 for cholera guinea pigs.

A special technique has been developed for demonstrating the presence of these serum lysins which is both quicker, more exact, and more delicate than the microscope or plate count method described at the beginning of this chapter. This special

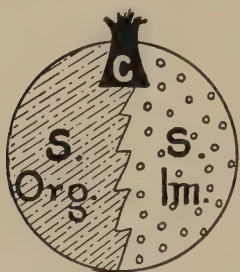


FIG. 110.—Diagrammatic representation of the combination of a given organism and the specific "immune body", held together by the "locking" complement.

The joining surfaces of the syphilis organism and the immune body formed against syphilis are matched to indicate the specific character of the immune body. After ZINSSER, Infection and Resistance, Macmillan.

technique utilizes four of the principles already discussed: (1) a lysin is a double not a single substance; (2) the complement part of the lysin is readily destroyed by heat and the immune part is not; (3) complement is but slightly or not at all specific in its binding power; and (4) complement combinations once made are stable. Let us briefly describe in a general way an early form of the lysin or complement-fixation test for syphilis in which the complement is combined or fixed (complement fixation test), and the reader may afterward locate for himself these four principles.

General Description of Complement Fixation Test.—A sample of the patient's serum is heated to 60° C. This destroys

the complement always present in blood or serum, but leaves in it the specific immune part of any contained lysin against syphilis. This (1) heated serum of the patient is put into a test tube with (2) syphilis organisms (a culture of syphilis organisms or a piece of syphilitic tissue such as the liver containing syphilis organisms) and (3) a limited amount of complement from normal blood, *e.g.*, a guinea pig. If the patient has syphilis, the immune part of the lysin in his serum will unite with the syphilis organisms, and both of these substances will be bound together by the guinea pig complement.

All this has been described before. The new and ingenious part of the test consists in adding at this stage red blood corpuscles to show whether or not the three substances have been bound together—whether or not the complement has been used.

To the test tube, therefore, are now added two more things which are themselves also capable of using the complement, and which need it to complete their combination: the red blood cells of a sheep, and the heated serum of another animal, a guinea pig, immunized against sheep cells and which therefore contains the stable immune part of the lysins the guinea pig has made against the sheep corpuscles. The guinea pig serum is heated to destroy its own complement; that means that these last added substances (sheep corpuscles and heated guinea pig serum) will have to depend on the complement already in the tube for their complete combination.

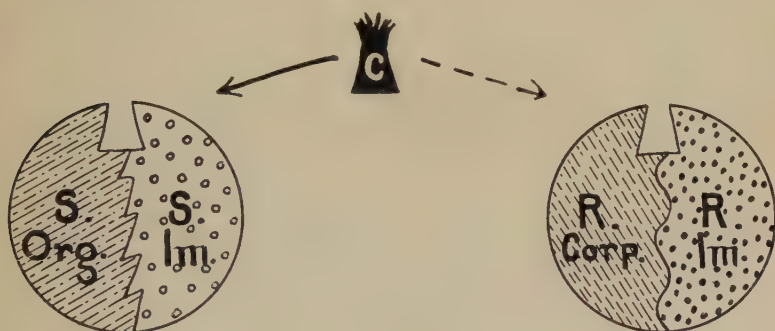


FIG. 111.—Complement making the left hand combination is not able to make the right hand one also. In such cases the red corpuscles will not be dissolved, and the tube will clear as the unbroken cells settle to the bottom. After ZINSSER, *Infection and Resistance*, Macmillan.

If the patient has syphilis, and, therefore, has the immune part of the lysin in his serum, the limited amount of complement present in the test tube mixture will all be combined in the first part of the above test, and, therefore, will not be free to unite with the red corpuscles and the specific immune part of the guinea pig serum or lysin which were added afterward. The red corpuscles will, therefore, sink unbroken to the bottom of the tube. In other words, the complement having made the first or syphilis combination, as indicated by the heavy arrow in (Fig. 111) above, cannot also make the red corpuscle combination indicated by the dotted arrow.

If the patient's serum does not contain the immune body for syphilis, the syphilis organisms alone will not be able to hold the complement in combination, and the complement will there-

fore be free to make the second combination with the red corpuscles indicated by the dotted arrow.

In this event the hemoglobin will be dissolved out of the red cells, the liquid becoming red throughout. And, having thus demonstrated that the patient's serum lacks the reacting lysins for syphilis, we conclude that he has not syphilis. Such coloration of the tube is therefore called a negative test or result for the

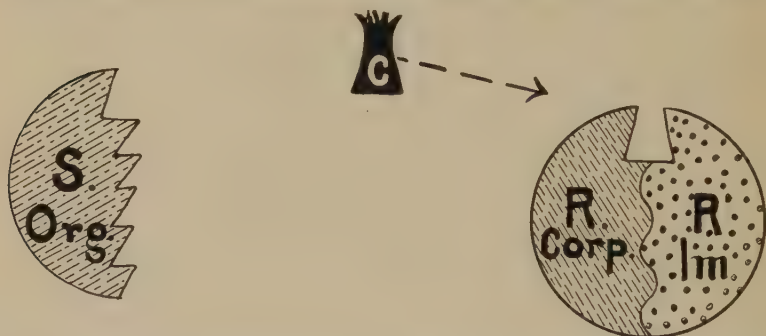


FIG. 112.—If the immune substance which helps hold the complement is lacking, the complement will be free to dissolve the red cells. A flushed or reddened tube indicates the lack of such specific immune substances in the patient's serum, and is termed, therefore, a negative test. After ZINSSER, *Infection and Resistance*, Macmillan.

disease organism represented in the first combination in the tube mixtures. A clearing tube, as described in the preceding paragraph, indicates on the contrary, that the patient did have syphilis and is therefore termed a positive test * or result. (Pl. IV.)

* The degree of reaction of any individual in a given infection may be gauged in a general way at least by the amount of antibodies produced. Since acute or serious infections would demand a plentiful supply of the specific antibodies, it is natural to consider a higher production of lysins, for example, as indicating a greater need for such lysins, and therefore implying a more serious or intense form of the disease.

Positive tests for syphilis often show a great variation in the amount of lysin present as judged by the red color changes in the Wassermann test (Pl. IV). Serum containing a high production of lysin would use up all the complement and therefore the tubes would remain absolutely clear. Serum low in lysin antibodies might not use it all, and would therefore leave some complement for combination with the red cells. The serum of such a patient would be described as weakly positive. In ordinary practice, four grades of positive results are usually recognized and the degree of each indicated by + or positive signs, +, ++, +++, and +++++. As a patient improves under treatment his reactions change from +++++ to + types and finally, on recovery, to wholly negative (—) results.

Such tests, it is evident, must be very carefully made. For example, if too much complement is put into the tube in the first part of the experiment, enough will be left over to make the second combination, and so a colored liquid will result—giving an apparently negative test—even when the patient has syphilis (Fig. 114).

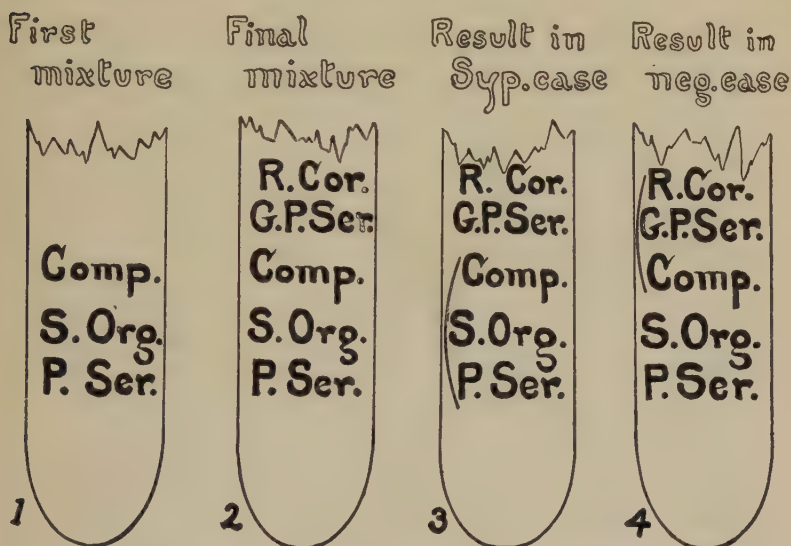


FIG. 113.—Tubes showing the substances used in the Wasserman test, the order in which they are mixed, and the fate of complement in negative and positive cases, as described in the text and in the preceding illustrations.

Modifications of the Wassermann Test.—The test just described gives in a general way an early form of the complement fixation test for syphilis, known as the Wassermann test. Many modifications have since been made in this test, such as the use of other kinds of red corpuscles; for example, in one method human red cells and serum from rabbits injected with human red cells are used to make the second part of the combination.

The most notable modification is perhaps one by Noguchi. Instead of the syphilitic organisms themselves may be substituted other reacting materials or antigens, such as heart muscle extracts (cow, guinea pig). These extracts (acting through their peculiar lipid or protein-lipoid content probably), make surprisingly

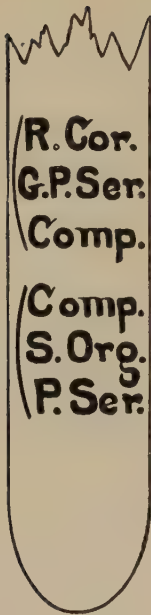


FIG. 114.—If an excessive amount of complement is present, there may be enough to make the red blood cell combination indicated by the upper brace in the tube, even though the patient had syphilis and the usual amount of complement was used in the first part of the procedure. This would lead to a false conclusion of "negative." This shows how carefully the measurement of the respective substances must be done and how important it is that such tests should be made by reliable laboratories.

satisfactory substitutes for the syphilitic material or antigen, as it is called. In certain stages of syphilis the results with these antigens correspond more nearly to clinical diagnoses than when the syphilitic material itself is used in the test.

Such substitutions make these tests seem much more complicated—and very much more difficult to describe in a non-technical way, but very often simplify greatly the preparatory work involved in such a test; for example, it is a much simpler matter to secure a heart muscle extract than satisfactory syphilitic material.

Descriptions of such tests sound complicated because we have really two overlapping tests made in one tube; the ingenious part of the test lies in using, for the second part of the test, a combination yielding color changes, so that one can judge by the visible results of the second part what did or did not take place in the first part of the experiment. This red corpuscle method of indicating the presence or absence of lysins is superior to the slower method of making, incubating, and counting agar plates (p. 154), not only in regard to time but also with regard to the delicacy and accuracy of the results obtained. Where it is difficult or impossible to cultivate the organisms under laboratory conditions, as with syphilis, this red corpuscle method is the only one that can be used.

Reliability of Complement Fixation Tests for Syphilis.

—In such tests, as with the tests for agglutinins or all other body reactions, early or incipient stages of disease are not always correctly diagnosed. If the tests fail in these advanced stages the clinical symptoms are usually strongly indicative. Other diseases

such as malaria, scarlet fever, leprosy, and sleeping sickness may interfere with the proper diagnosis of syphilis and misleading

positive results may be obtained. Treatment with certain curative preparations containing mercury and salvarsan may cause negative results, even though the patients are not yet cured; and after such treatment, repeated negative tests for a long period (three at intervals of six months) should be obtained before the

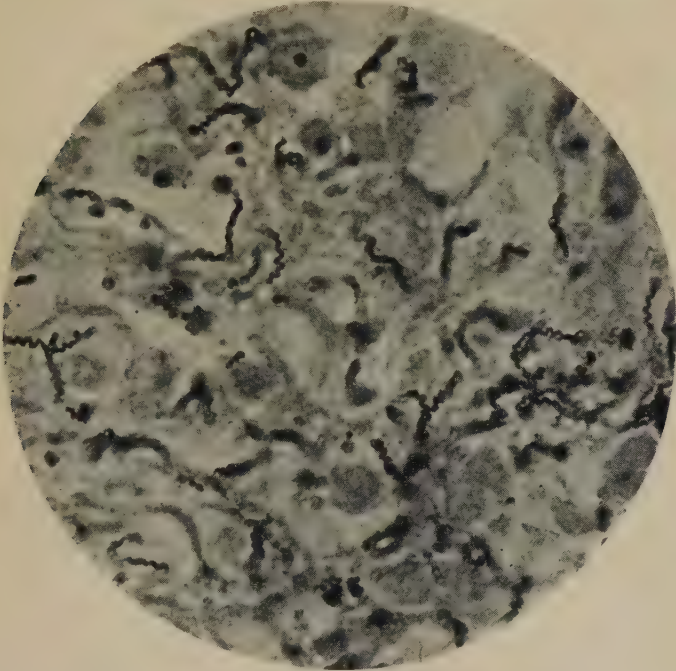


FIG. 115.—Human brain tissue, (congenital syphilis) containing syphilis organisms, *Treponema pallidum*, (x 960). LEO M. POWELL.

individual is considered free from disease. Alcohol, too, may weaken the reaction in such tests, and anæsthetics may give false positive reactions.

Nevertheless, the many years of experience with this test, indicate that the tests of the blood or spinal fluid if properly carried out give accurate results in 80 to 88 per cent. of the cases in all except the early stages (first five to six weeks). Some workers claim a still higher per cent. of accuracy—90 per cent. or more in all active general infections.

Other Diseases Diagnosed by Complement Fixation Tests.—Complement fixation tests are used in several other diseases. In each the serum of the patient is tested with known bacteria—one or more strains of the suspected bacteria being used in various forms: alcoholic and ether extracts of dried bacteria, emulsions of centrifuged residue of washed cultures, etc.

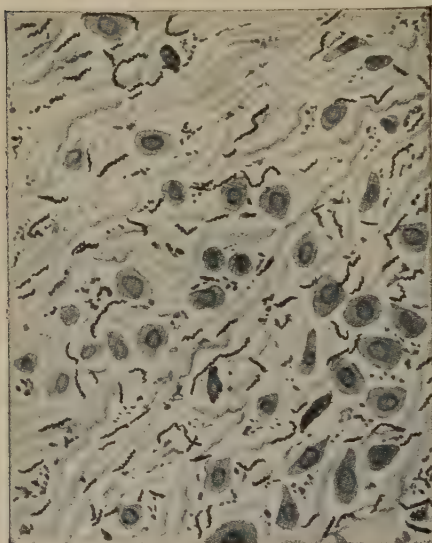


FIG. 116.—Disintegrating effects of the syphilis organism, *Treponema pallidum*; the liver substance (new-born child) in which these organisms are living is wholly unrecognizable. (x 880). ADAMI AND MC CRAE, Text Book of Pathology, Lea and Febiger.

In typhoid infections the complement fixation test is used mainly to corroborate the agglutinin results. In whooping cough, the pertussis bacillus, considered by many the causal organism, has given positive reactions in about 50 per cent. of a large number of cases in which the serum of the patients was tested by this method.

For glanders, the New York City Health Department finds the complement fixation test less reliable than the agglutination test in very acute cases because the agglutinins are the most abundant antibodies in the

early stages. For more chronic cases the complement fixation test is considered best. Used together, it is claimed that these two will give a correct diagnosis in 99 per cent. of the cases. The complement fixation test is used by most workers to corroborate the agglutinin test and has not supplanted it.

In meningitis, the results by the complement fixation test are satisfactory, but the diagnosis of meningitis can be too satisfactorily and quickly made by microscopic and chemical examination of the spinal fluid to make it worth while to fully develop

this method. This test is, however, used when it is desired to distinguish the types of meningococcus from each other.

In tuberculosis, the outlook is very encouraging; investigators showing that while the complement fixation test does not show up incipient cases, it does give positive reactions in "75 per cent. to 95 per cent. of clinically active pulmonary" cases. It is also stated that, unlike the much-disputed tuberculin reaction, it gives positive reactions in active cases only and not in healed lesions. Contrary to the statements of early investigators in this field, this test is specific, and gives a positive reaction in tuberculosis only.

The complement fixation test is an accepted test in gonorrheal infections. Though not reliable in the early stages (before the fourth week) and though it sometimes fails to give positive reactions in certain types of gonorrheal infection, such as acute vulvovaginitis, when positive reactions are obtained they are considered reliable, if it is remembered that the positive reactions may continue one or two months after a cure has been effected. It may well be, that as encouraging data can be given for these five diseases as for syphilis, when a corresponding amount of work has been devoted to them. Recent work indicates the probability that similar tests may be applicable to malignant tumors.

Complement Fixation in Standardizing Serum.—An important though relatively recent use of the complement fixation test is made in standardizing immune serums, measuring the strength of serum by the power of varying dilutions or amounts of the serum to fix or combine with known amounts of complement and standard preparations of bacteria.

After these three substances (serum, complement and bacteria) have been properly diluted and mixed, red corpuscles and an immune red corpuscle serum are added—the usual complement fixation test. The principal serum tested in this way is meningitis antiserum. (See also p. 124 and p 138.)

Protein Tests.—The complement fixation method affords a more delicate test than the precipitin tests for detecting foreign proteins or differentiating between various proteins. The unknown protein is tested against the serums of different animals each injected with a special protein. The method is essentially that already described for diagnosing disease, the results being

made visual in the same way by later additions of red corpuscles and an immune red corpuscle serum. By this method it is possible to detect foreign proteins when the amount present is too small to be visible by the simpler precipitation methods.

STUDY SUGGESTIONS

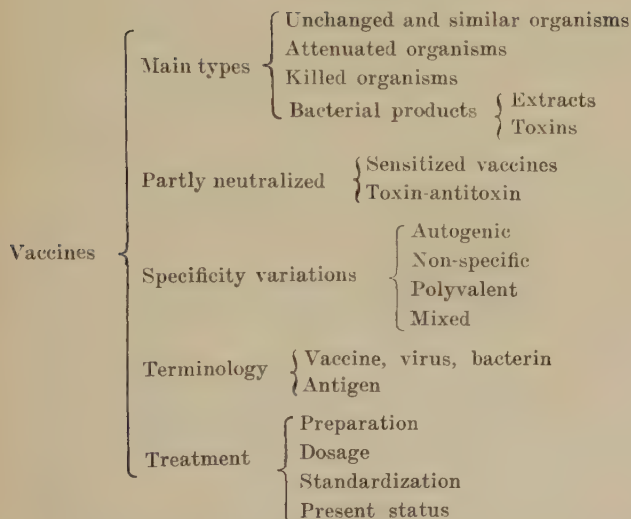
1. What are lysins? Give four diseases against which lysins are important aids.
2. Explain why we think lysins are "double" substances.
3. Show how lysins may be used in identifying samples of blood.
4. Contrast complement and the immune part (immune body or antibody) of lysins in specificity, resistance to heat, and their presence and absence in normal and immune individuals.
5. Consult an advanced text on bacteriology or pathological diagnosis for a more complete description of the Wassermann test and write up a negative and a positive test as simply and as briefly as you can.
6. Compare the Wassermann test with a very different test such as the "colloidal gold" test for syphilis, showing the difference in theory or principle in each test.
7. Among the directions for treating an animal to cause it to produce lysins for use in blood tests we find:
 - (1) Wash and centrifuge the blood cells to be injected in salt solution.
 - (2) Inject 5 to 20 c.c. of the washed blood cells at intervals of 5 or 6 days.
 - (3) Blood may be drawn six or more days after the third injection.

Answer as many of the following questions as you can:

- (a) Why are the blood cells washed?
- (b) Why are three sets of injections used instead of one?
- (c) If it is desired to continue to obtain blood through a period of several months what supplementary direction should be added to the above?

CHAPTER IX

VACCINES



THE oldest field of bacteriology is that of vaccines, for the control and prevention of disease by the direct use of micro-organisms began centuries ago. But despite the fact that the period of investigation along this line is measured by centuries, while that of antitoxins and other phases of immunity is measured by decades only, the most important modifications and advances in vaccines have been made in the last few years.

Early Methods of Transferring Smallpox.—Even centuries ago, several of the Asiatic peoples purposely transferred smallpox by such practices as wearing the clothing or sleeping in the beds of people recovering from light cases of smallpox. The Chinese transferred smallpox still more directly, collecting pus from smallpox pustules on bits of wool, etc., and placing them in the nostrils of the person who wished to contract the disease. In Turkey, a still more direct method of transferring smallpox

organisms was practiced, the pus from a mild case being inserted into or under the skin.

An individual inoculated in any of these ways from a mild case was likely to have a similarly mild attack. While this did not always prove true, the risk was a slight consideration in an age when the only choice was—not whether one would contract smallpox or not—but when one would have it; under such conditions it was wiser to endeavor to contract a mild form of the disease, than to take one's chances in the next epidemic.

Control by Inoculation.—The Turkish method of direct inoculation was introduced into Great Britain in 1718 by an English woman, Lady Montague, who had had one of her children inoculated during a short residence in Turkey. This method was continued in England for over a century—until forbidden by a special act of Parliament, owing to the perfection of Jenner's new and more reliable method of preventing smallpox. (1840)

In our own country the inoculation method was practiced much longer, however; as late as 1863 the people of Richmond, Va., were besought by a house to house canvass to have their children inoculated that the scabs containing smallpox organisms might be collected to provide material for the inoculation of soldiers in the Confederate army.

Protection by Using Similar Organisms.—There was current among English dairy people the opinion that those who had had cowpox did not later develop smallpox. Acting on this idea, a farmer named Jesty, in 1774, inoculated his wife and two sons with pus from a cow having cowpox, and to this he attributed their later immunity to smallpox.

Real proof, however, that cowpox protects against smallpox was first given by Jenner, a doctor, who inoculated with smallpox pus, ten people who had previously had cowpox, the interval between the earlier attack of cowpox and the inoculation with smallpox ranging from nine months to fifty years. Not one of the ten contracted smallpox. For further proof, in 1796, Jenner inoculated a boy with pus taken from the hand of a dairymaid who had become infected with cowpox. Six weeks later, and again several months afterward, Jenner inoculated the boy with

real smallpox pus, but both times the boy proved resistant to the smallpox thus inoculated.

The explanation accepted for the resistance to smallpox in all these instances, is that cowpox organisms are so like smallpox organisms, that the body reacts against them in practically the same way as against smallpox, and therefore, each person recovering from cowpox has in his body reacting substances that fully protect against the smallpox organisms when he is later exposed to smallpox.

Since in these cases the infectious material came from a cow, it was called vaccine (from *vacca*, a cow). The original vaccines were (1) living organisms, and (2) cultivated in the body of a living animal, the cow. It will be interesting to trace in the following pages the changing meanings attaching to the term vaccine.

Protection by Weakened Organisms.—Cowpox and smallpox organisms may be considered as similar organisms. More general, however, is the feeling that cowpox is a modified or attenuated form of smallpox—that the disease was originally a human disease which has been transferred to cows by human contact and the less virulent character of cowpox is due to the fact that the original smallpox organisms have been weakened by the period spent in the cow's tissues. (See p. 63.)

It was Pasteur, however, who first developed a method of weakening or attenuating organisms by laboratory procedures, so that they might be used as vaccines.

In 1880, while Pasteur was working on fowl cholera, by an accident some hens were inoculated with an old culture of fowl cholera organisms made a few weeks before, instead of a fresh culture but a day old such as he had been using for that purpose. The hens sickened, but contrary to all of Pasteur's previous results, afterward recovered. Later, when these recovered hens were inoculated with fresh cultures of fowl cholera, they proved to be immune while the control hens died.

Pasteur properly interpreted this as indicating that the hens survived because they had been sufficiently irritated by the age-weakened organisms to produce enough reacting substances for full protection against the fresh, vigorous organisms used in the later or second inoculation.

It was essentially the same situation as when the cowpox organisms were used to protect against the more virulent small-pox organisms. The only difference was that the fowl cholera organisms had been weakened by unfavorable laboratory conditions, while the cowpox organisms had been affected by the conditions met in the living tissues of the cow.

Pasteur and his associates included in their study of weakened organisms, the effects of other laboratory conditions besides age: unfavorable temperatures, chemicals, etc. Pasteur's best known contribution in this line, however, was made with rabies, the rabies organisms being attenuated by drying. In this case, the organisms themselves not having been isolated, they were weakened by drying the brain (medulla) and spinal cord of rabid animals, as it was found that these tissues contained the organisms.

With such dried tissues Pasteur demonstrated that by gradually using organisms that were less and less weakened (dried for relatively shorter periods), he could protect dogs from rabies if they were later bitten by rabid animals or inoculated by him with saliva or brain tissue containing rabies organisms. He also proved that it was possible to protect dogs in the same way even if they were bitten or inoculated before he began treatment with the weakened organisms. The explanation is that rabies organisms develop very slowly, and therefore, before the few organisms entering when the dog is bitten can multiply sufficiently to produce rabies, the dog forms sufficient protecting antibodies against the numerous weakened organisms given in the series of inoculations. This method is now commonly used to protect people who have been bitten by rabid animals, but it was first tried by Pasteur in 1885, on an apparently hopeless case, a little boy terribly bitten (hand, legs and thighs) and covered with blood and saliva. Although the child was not brought to Pasteur until two and one-half days after being bitten, Pasteur undertook the case, beginning the treatment with spinal cord dried (Fig. 117) for fourteen days. On the tenth day he gave the last inoculation with spinal cord dried but one day. The successful outcome of this and another apparently hopeless case, in which treatment had been deferred for six days, brought Pasteur international reputation, (Four children from the United States were sent by

a New York newspaper to Pasteur for treatment) and firmly established the use of attenuated organisms as vaccines. Now our large cities have "Pasteur Institutes" or similar divisions of the Health Department for developing immunity to rabies by the use of weakened organisms.

Killed Organisms as Vaccines. —

As shown above, weakened organisms arouse much the same reactions as ordinary live organisms. In the first dose given in rabies treatment, most of the organisms (Fig. 118 a, b.) have doubtless been killed by the long drying period just described. These dead organisms contain or are composed of much the same irritating substances as the live ones, and so

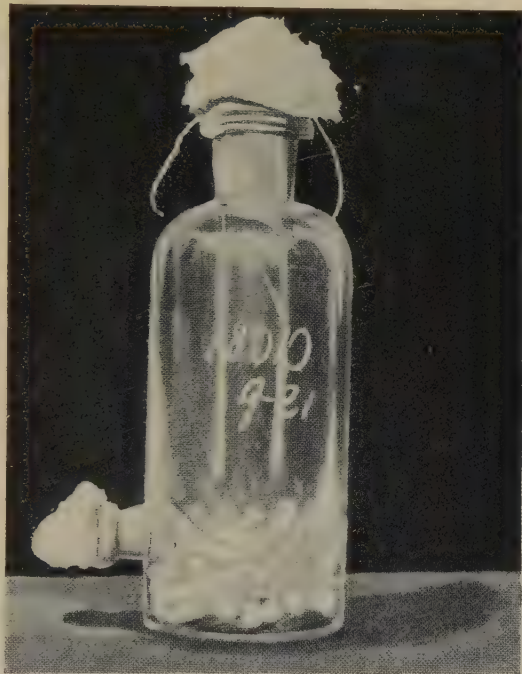


FIG. 117.—Method of drying sections of spinal cord before injection as vaccine; two pieces of cord are shown suspended over a water-absorbing chemical. STIMSON, U. S. Bureau of Animal Industry.

begin the stimulation of the same reactions which the live organisms induce much more vigorously. Each succeeding dose in the series of inoculations given in treated rabies, contains relatively more and more live organisms; this is necessary because dead organisms alone do not arouse sufficient body reactions.

In many diseases, however, dead organisms are sufficient. If the bacteri effects are due mainly to the toxins which they

form, these same toxins are sufficiently represented in the broth in which the bacteria are grown. If the effects are due to the disintegrating proteins of the bacterial cells, this disintegration may take place even more rapidly when dead bacteria are used than when live cultures are inoculated. There is often a great advantage in using killed organisms, for though the protecting substances they excite may not be so lasting, it is possible to measure the doses much more accurately and to predict their effect much more definitely. It is possible also to give with safety, relatively much larger doses than when live organisms are given, for the effects of killed bacteria are limited, since they can not multiply in the body.

In the United States the most widely used vaccine made of killed bacteria is typhoid vaccine. *Staphylococcus* vaccine (boils, skin eruptions) probably ranks second.

Bacterial Extracts as Vaccines.—Bacterial extracts (alcoholic or glycerine extracts or emulsions of broken or disintegrated bacteria) may be used instead of killed bacteria as vaccines. All vaccines—even living ones—contain some disintegrated bacterial substances. Bacterial extracts have been advocated for two reasons: (1) Such extracts contain relatively more of the toxins or other characteristic irritating substance. Since all foreign protein is irritating when injected in this way, it would be a great gain if only the necessary irritating substances could be given. (2) Another reason advanced is the more rapid absorption * of such bacterial extracts or partly digested bacteria. This absorption, however, may be too rapid; instead of causing the quicker response desired, it may overpower the body before the responses are aroused. Little if any practical use is, as yet, made of such bacterial extracts.

Toxins as Vaccines.—It is not the custom to speak of toxins as vaccines, but there is no real reason why toxins should not be included as a fifth of the series of substances we have been

* This difference in the absorption rate is illustrated by Vaughan's experiments with three types of preparations of the colon bacterium—live bacteria, killed ones, and his specially prepared extract (split-proteins) of the colon organism. The guinea pigs injected with the living organism showed no symptoms for five to twelve hours, and death occurred in one and a half to two days; inoculations with the killed bacteria caused death in six to twelve hours, and the guinea pigs receiving the extract died in thirty to sixty minutes.

discussing: (1) Live organisms; (2) attenuated organisms; (3) killed organisms; (4) extracts; and (5) toxins. (See also p. 98.)

Such toxins are ordinarily obtained by filtering the broth cultures of the desired bacteria, *e.g.*, diphtheria. These filtrates, loosely called toxins, contain much besides the soluble toxins—such as the broth itself and other products of ordinary growth and metabolism.

When these toxin filtrates are injected into the body, the soluble toxins are carried by the blood stream throughout the body, just

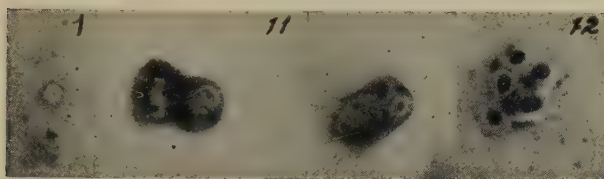


FIG. 118 A.—Rabies organism or Negri bodies. Nucleated bodies from cultures are shown in 1, 11 and 12.

as they would be distributed from a disease focus in the body, *e.g.*, the throat patches in diphtheria. Toxins are given horses to cause them to produce antitoxin for diphtheria or tetanus. It is only recently—to prevent diphtheria—that toxins have been injected into human beings to stimulate their own production of antitoxins.

“Sensitized Vaccines” and “Toxin-Antitoxin.”—A vaccine is sometimes modified by allowing it to stand for some hours mixed with the antiserum formed in response to that particular organism, *e.g.*, Shiga’s dysentery organism. Such modified vaccines are termed “sensitized vaccines” or “sero-vaccines.” Much the same result is secured by injecting a vaccine and its respective antiserum simultaneously.

Antitoxins may similarly be used to modify toxin, in the same way as antiserum and its respective vaccine are combined. (See p. 98.) Diphtheria toxin, when it is used to produce immunity to diphtheria, is thus modified by the addition of antitoxin. This combination is called “toxin-antitoxin.” In both the “sensitized vaccines” and the “toxin-antitoxins,” larger doses may be given than if simple vaccines or simple toxins are used. As explained on

page 99, the antitoxins or antibodies apparently do not neutralize part of the vaccine, leaving the balance unaffected, but instead tend to distribute themselves over the whole amount of toxin or vaccine weakening every particle of it, thus making it possible to give a larger dose of stimulating but somewhat less irritating material. The desired body reactions are, therefore, aroused with much less "shock" or other undesirable symptoms.

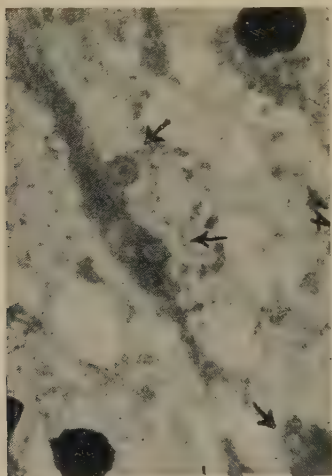


FIG. 118 B.—The arrows indicate the Negri bodies seen in a brain film or smear. (The large black bodies at the corners of No. 14 are nerve cell nuclei.) NOGUCHI, *Journal of Experimental Medicine*, Rockefeller Institute.

Autogenous Vaccines.—

Since there are many closely related species and even different varieties of some organisms, it is evident that a person vaccinated with one species or variety of a bacterium, such as streptococcus, may not gain full protection against a related species or variety. Sometimes, therefore, an effort is made to secure the individual's own variety of organisms, and use that to make the vaccine and so bring about his recovery. This takes time, for it is necessary to spread the pus, nasal excretion or other material containing the organisms, out on a special medium (*e.g.*, agar plates), and wait until

the organisms have developed, to secure the organism responsible for the infection. Broth or agar cultures are then made of the organisms thus isolated; these cultures are examined for purity, type, etc., and also tested for strength (See p. 183) before they can be used as vaccines. Such preparation takes several days at least, a delay advisable only in chronic or mild cases. In acute cases, the delay may not be compensated for by the difference between the ordinary laboratory or stock organisms and the individual's own variety. In streptococcus, pneumonia and similar infections, therefore, a stock vaccine is sometimes given until the individual's own variety can be isolated and made into a vaccine—an autogenous vaccine.

Treatment with Non-Specific Vaccines.—Beneficial results from closely related organisms (such as cowpox in preventing smallpox) have been already discussed, but benefit from the use of widely differing protein substances—not necessarily bacterial in their origin, for example, albumose in typhoid fever, is

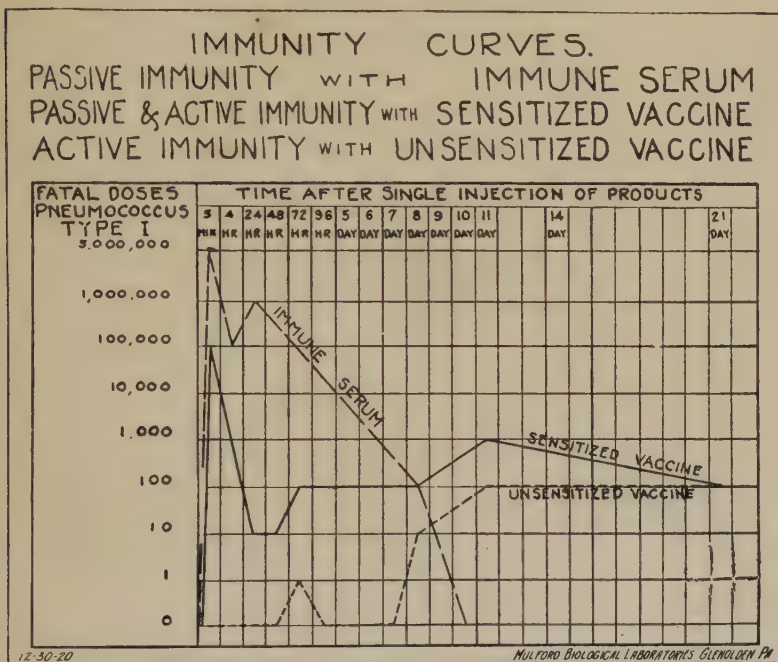


FIG. 119.—A comparison of the results obtained with two types of pneumococcus vaccines with mice. The sensitized vaccine, like immune serum, gives high initial immunity; and it arouses, later, a higher active immunity than ordinary (unsensitized) vaccine. CORSA, H. K. Mulford Co.

more difficult to understand. More than one factor is doubtless involved in this protective response; an important one doubtless is the effect of such injected substances, as albumose, on the tissues which form the various antibodies, causing a rapid increase of typhoid antibodies and white corpuscles. The fact that the amount or strength of these responses is greatest if the injections are begun some days after the disease organisms begin to develop in the body (about the tenth day in typhoid, for example), seems

to indicate that such increases are due to the liberation of antibodies already formed—that the response is not an increase in specific reactions but a stimulation of the tissues concerned, to release the antibodies already formed. Such non-specific results do not lessen the value of the ordinary specific vaccines in common practice, however, for in most cases specific stimulation is necessary to increase the actual formation of protective substances. Neither do the results with non-specific substances war-

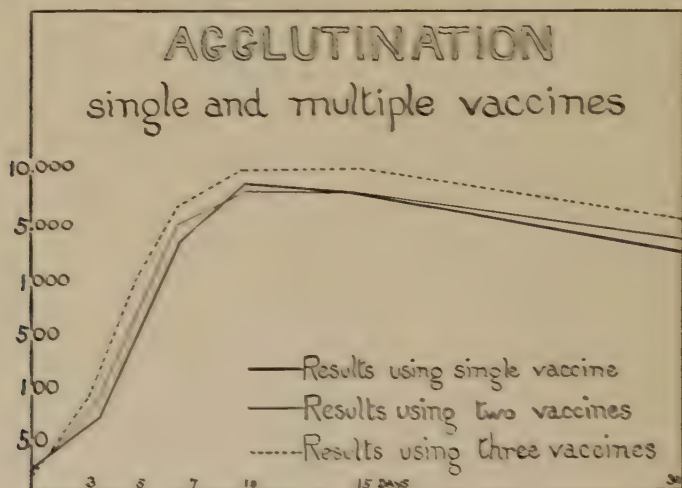


FIG. 120.—Result with one, two, and three vaccines (typhoid alone, or typhoid with coli or pseudotubercular organism, or both) with regard to the reaction aroused against typhoid, justifying the use of mixed vaccines. Redrawn from Hinton (after Castellani), *Journal of Immunology*.

rant the general or indiscriminate use of mixed vaccines (p. 179) or of other non-bacterial or "non-specific" proteins, for the increase in the irritating material thus injected has its attendant disadvantages (See p. 192 and p. 193).

Polyvalent Vaccines.—Two or more strains, *e.g.*, of staphylococcus, may be mixed together and used as a vaccine, the design being to be sure to include the particular strain against which the individual needs increased resistance, without the delay caused by preparing an autogenous vaccine. In such polyvalent vaccines there is a great increase in the amount of foreign protein injected.

and a corresponding increase in the work to be done by the body when it can least afford such expenditures of energy.

Mixed Vaccines.—In certain diseases where the causal organism is not definitely known, as in colds, or where more than one organism is frequently associated with the disease, as in influenza, a mixed vaccine is sometimes given. In influenza, such a mixed vaccine may contain influenza, streptococcus and pneumococcus organisms. Such treatment is open to the same objections as in the case of “polyvalent vaccines.”

Slightly different, however, is the simultaneous injection of two or more different vaccines each of which is necessary to protect against a possible infection and where, therefore, each type of body response is needed. In the recent war, such protection had to be developed in a very short time, and it was necessary to give the requisite vaccines simultaneously. Our own army used for such purposes a triple vaccine containing typhoid, paratyphoid and dysentery organisms. In the Russian army a tetra-vaccine was used, cholera being the fourth kind of organism included.

Vaccine, Virus and Bacterin.—The original vaccines were live organisms which were grown in the body of a living animal. Most of our vaccines are now cultivated in laboratory media and are used after they have been killed. For these killed organisms the word bacterin has been tried but has never come into popular use. Though dead and though cultivated under very different conditions, they are after all like the original smallpox vaccine in the one essential—they cause the required body reactions.

Virus is a term used by some writers to designate living vaccine material and vaccine for killed forms. This is a shifting of the term vaccine which seems unjustified, for if any distinction of this kind is to be made, the word vaccine should be retained for the class of living vaccines in which smallpox vaccine, the first known vaccine, still belongs.

The word virus is more justifiably used in another sense—to indicate the infectious organism when the speaker does not know a more definite name for it, because the organism has never been seen (as in smallpox), or because its proper classification is still unsettled (*e.g.*, the globoid Flexner-Noguchi organism in infantile paralysis).

Because of all these variations in meaning or usage, it seems

much wiser to avoid over emphasis on the terminology, and accept the term vaccine in the broadest possible sense—for organisms or their products which are capable of arousing protective body reactions; this is especially desirable as the many millions of people vaccinated with both live organisms (smallpox) and dead organisms (typhoid) during this recent war have used for both processes but the one term, vaccination.

Antigens.—Any substance which stimulates the production of antibodies is called an antigen, (*anti*, against; and *gen*, producing); all vaccines (live cowpox organisms, killed typhoid bacteria and diphtheria toxin)

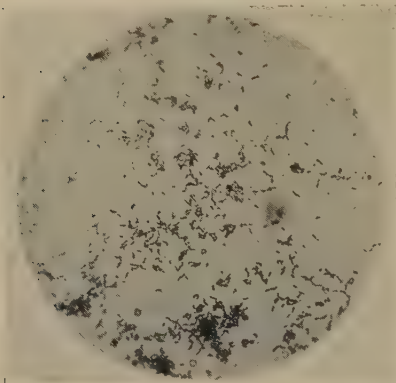


FIG. 121.—*Fusiformis acnes* (*Bacillus acnes*), a diphteria-like organism, growing best with minimum amounts of oxygen (x 700).

are, therefore, antigens. In many laboratories, however, it happens that the term antigen is rarely used for the organisms in reference to their part in producing these antibodies in a given individual, but rather for the organisms (or substitutes for them) in relation to their power to combine with the antibodies present in laboratory samples of an individual's blood. In other words the

term antigen is much more commonly used in connection with diagnosis—with tests for disease—than in connection with the prevention or treatment of disease. For example, we speak of the killed typhoid bacteria or the vaccine used to vaccinate against typhoid, but we use antigen to indicate the typhoid organisms when we test a patient's serum for agglutinins. The expression "syphilitic antigen" may mean any of the several substances—syphilis organisms, syphilitic tissue or even non-syphilitic substances such as heart extracts—which will unite with the syphilis antibodies an individual has produced and so show up in a laboratory test, such as the complement fixation test. (See p. 160.)

Preparation of Vaccines.—The organisms used in making

vaccines may be grown in broth or agar. They may be killed by disinfectants (carbolic, tricresol, chloroform, alcohol, etc.), or they may be killed by heat (about 55° C.); if chemicals are also used in preparing heat-killed vaccines, they are added mainly as preservatives. In addition, the organisms may be finely ground or pulverized; for the lipo-vaccines, they are ground in specially constructed apparatus for many hours. The organisms are finally made up in liquid form—as normal salt suspensions or as lipo-vaccines, in which case they are combined with lanolin and cotton seed oil. The oil in the lipo-vaccines is thought to delay absorption, making one small dose serve as the equivalent of several repeated doses with consequently better production of antibodies. Our army in the recent war used mainly the single-dose lipo-vaccine, while the navy used the old three-dose saline suspension, as not enough of the newer lipo-vaccines was avail-



FIG. 122.—Syringe for injecting vaccines. In some syringes the tube is graduated (see Fig. 58) to guide the physician in calculating the amount to be injected.

able for both the army and the navy, and the complicated book-keeping where three doses were required was more difficult in the shifting life of an army in preparation, than in the navy. The army has since resumed the old three-dose saline vaccine. Most vaccines can be kept at low temperatures for three months or even longer without great loss in strength. Lipo-vaccines have a longer "keeping" period.

Dosage.—Vaccine dosage varies greatly, both in number of doses, and in the size of the dose. In preventive treatment, vaccines are usually limited to one to three doses, as in typhoid. When given as a curative measure, as in rabies or staphylococcus infections, the doses may be repeated throughout a considerable period, for weeks or even months—until definite body reactions have been aroused as indicated by special tests for the increase of antibodies (*e.g.*, opsonins against staphylococcus), or by the patient's physical symptoms. Most vaccines are given with intervals ranging from two to seven days, though three or four days are probably the best intervals—at least for preventive work.

TWENTY-ONE DAY SCHEME OF RABIES VACCINE TREATMENT

Day	Days Cord Dried		No. of Injections	Adults	Children	
		Face Cases			6 to 10 yrs.	1 to 5 yrs.
1	8-7-6	8-7-6	2	3 c.c.	3 c.c.	3 c.c.
2	8-7-6	8-7-6	2	3 c.c.	3 c.c.	3 c.c.
3	5-4	5-4	2	3 c.c.	3 c.c.	3 c.c.
4	5	5	1	2 c.c.	2 c.c.	2 c.c.
5	4	4	1	2 c.c.	2 c.c.	*1½c.c.
6	4	4	1	2 c.c.	2 c.c.	*1½c.c.
7	3	3	1	2 c.c.	*1½c.c.	1 c.c.
8	3	3	1	2 c.c.	*1½c.c.	1 c.c.
9	5	2	1	2 c.c.	2 c.c.	2 c.c.
10	4	4	1	2 c.c.	2 c.c.	2 c.c.
11	4	4	1	2 c.c.	2 c.c.	2 c.c.
12	3	3	1	2 c.c.	2 c.c.	*1½c.c.
13	3	2	1	2 c.c.	2 c.c.	*1½c.c.
14	4	4	1	2 c.c.	2 c.c.	2 c.c.
15	4	4	1	2 c.c.	2 c.c.	2 c.c.
16	3	3	1	2 c.c.	2 c.c.	*1½c.c.
17	3	2	1	2 c.c.	2 c.c.	*1½c.c.
18	4	4	1	2 c.c.	2 c.c.	2 c.c.
19	4	4	1	2 c.c.	2 c.c.	2 c.c.
20	3	3	1	2 c.c.	2 c.c.	2 c.c.
21	3	2	1	2 c.c.	2 c.c.	2 c.c.

2/10 per cent. phenol used as a preservative instead of glycerine.

*Face cases receive adult doses.

FIG. 123.—Copy of treatment now given in the New York City Health Department. Face cases may be given treatment with spinal cords dried but two days, or the whole twenty-one day treatment may be repeated for such cases. (Bureau of Laboratories, Department of Health, City of New York.)

The doses are often small in actual bulk, for example, they may be as small as 1/1000 of a milligram (1/1000 mgm.) in the initial dose of tuberculosis vaccine (tuberculin). Most of us can visualize better the bulk of the larger but still minute dose used for smallpox vaccination; small as it is, less than one-fifth of it is composed of the causal organisms themselves. (See next paragraph.) A clearer idea of what the dosage means to the body is gained from the number of bacteria in a dose. A dose, usually, is somewhere between two million and five hundred million bacteria, and it often is as high as one billion or even ten to thirty billion. In most cases, vaccines are finally diluted so that they range from one hundred million to five hundred million per cubic centimetre, and the dose is administered in cubic centimetres or appropriate fractions thereof.

Standardization of Vaccines.—

In the use of some vaccines, such as smallpox vaccine, where the organisms themselves cannot be seen to be counted, the doses are measured in a rather crude way, mainly by bulk or weight. In making smallpox vaccine the pulpy exudate containing the organisms is scraped off the body of the calf and ground up with four times its bulk of glycerine, water, and carbolic acid. This glycerinated mixture is distributed for use in tiny glass capillary tubes from which it is expelled in minute amounts to vaccinate human beings. The potency of the vaccine is proven before the preparations are sold or distributed, by showing that a given number of vaccinations, usually fifteen, is 100 per cent. successful when used on children who have never been vaccinated.

Vaccines are sometimes graded more definitely by weight with regard to the organisms they contain. The bacteria used in

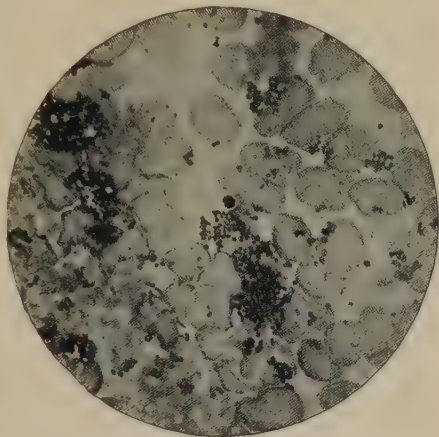


FIG. 124.—Part of a slide with a mass of bacteria (staphylococci) at the end of a blood film, showing why this method of estimating the number of bacteria in a vaccine often results in an underestimate (100 to 200%) (x 800). Muir, *Journal of Pathology and Bacteriology*.

making the vaccines represent fixed amounts by weight per cubic centimetre; in the lipo-vaccines recently used in the army, meningococcus organisms represented 1.8 milligrams, and pneumococcus, 2.5 to 3.5 milligrams, respectively.



FIG. 125. —Small capillary tubes, fused shut at the upper end, standing in the container used for filling. The lower open end of each comes into contact with the vaccine as it runs out of the bottom of the large central tube. See next illustration.

Usually, however, the strength of a vaccine is determined much more definitely by an actual counting process. This may be done by microscopic determination of the number of bacteria present in a given bulk, *e.g.*, one cubic centimetre. The bacteria are usually determined in one of the following ways: (1) By mixing equal amounts of blood and the bacterial culture; for since we know the number of red blood cells in a cubic centimetre, the proportion of bacteria and blood cells in the mixture on the slide tells us the number of bacteria per cubic centimetre; (2) or, by counting the bacteria alone, using the well-known blood-count slides, and counting the bacteria by the aid of the markings on such a slide.

It is not necessary to count the bacteria in each batch of

vaccine. Once the bacterial content of a series of vaccine dilutions has been determined, it is easy to make up a set of tubes of the same range in turbidity, using for this standard turbidity set, some finely divided substance such as silica. Once we have determined the number of bacteria per cubic centimetre corresponding with the silica standards, the determination of the strength of a new lot is very easily determined, by a simple "matching up" process.

Present Status of Vaccines.—At present the results with some of the prophylactic vaccines—in preventing disease—are on a more unchallenged basis than can be claimed for the curative vaccines. In our own country smallpox, typhoid and rabies vaccines (Fig. 128) easily rank first. Diphtheria vaccine, for “toxin antitoxin” may be so called (See p. 102), has already

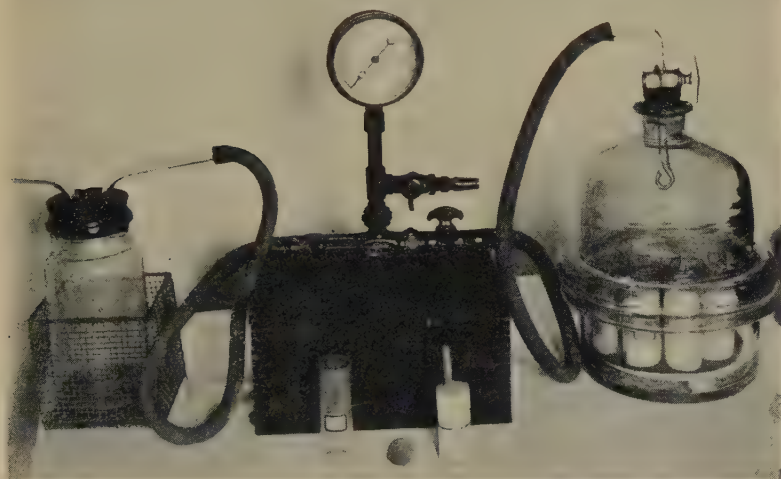


FIG. 126.—Ingenious device for filling the capillary tube shown in the previous illustration. Several of the containers are placed in the covered jar on the right. The air is drawn out of capillary tubes by establishing a vacuum. The vaccine in the bottom of the small containers is forced up into each of the capillary tubes by pressure and the end is fused shut. Bureau of Laboratories, Health Department of the City of New York

established its value beyond question, especially for young children. The dysentery vaccines (paratyphoid and Shiga's dysentery), and the vaccines for plague, and cholera, have shown their value in emergencies, such as epidemics or crowded war time conditions.

There is still great diversity of opinion with regard to the value of the vaccines used in preventing or in treating infections of the respiratory tract, such as whooping cough, pneumonia, influenza, mouth infections, and colds. This is partly explained by the fact that many different organisms are usually present in

the areas affected in each of these diseases. Persistent congestion of such areas as the frontal sinuses, the ears, etc., may resist vaccine treatment because such areas are too poorly supplied with blood to be greatly benefited by ordinary amounts of the antibodies formed against the vaccine.

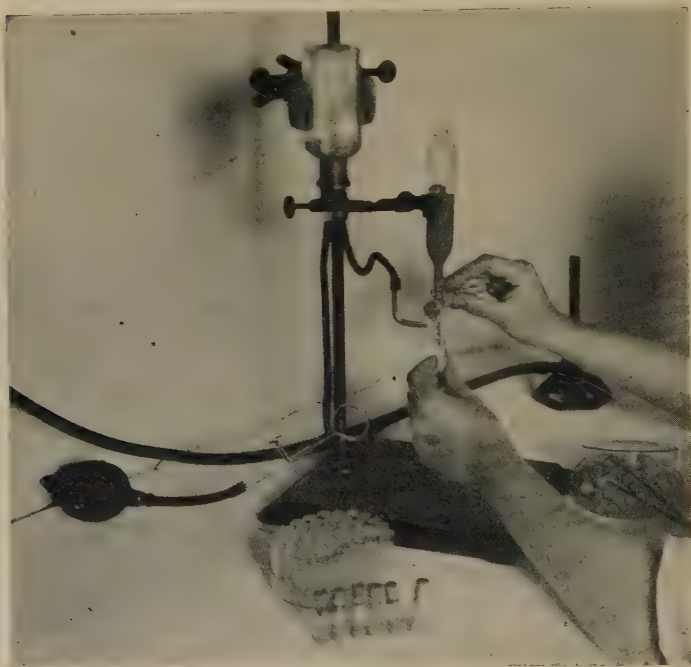


FIG. 127.—Filling small bottles with finished vaccine. Bureau of Laboratories, Health Department of the City of New York.

Vaccine treatment for meningitis has not been successfully established. Curative effects with vaccines are most marked in chronic or sub-acute diseases, such as staphylococcus infections, of which there is a wide variety, ranging from mild cases of skin infections (acne [Fig. 121]) and persistently recurring boils to chronic osteomyelitis. For streptococcus and gonococcus infections vaccines are much used; they are considered helpful in

treating such conditions as gonorrheal joints and other localized infections or inflammations.

Vaccines are still used by some physicians in treating tuberculosis, the advantages claimed being that tuberculin vaccine promotes the healing of existing lesions and *lessens* the tendency to the characteristic relapses in tuberculosis. As Park advises, users should realize that such a substance "is not a cure, and should be employed as an addition to other recognized methods of treatment." (See discussion in Anaphylaxis chapter following.)

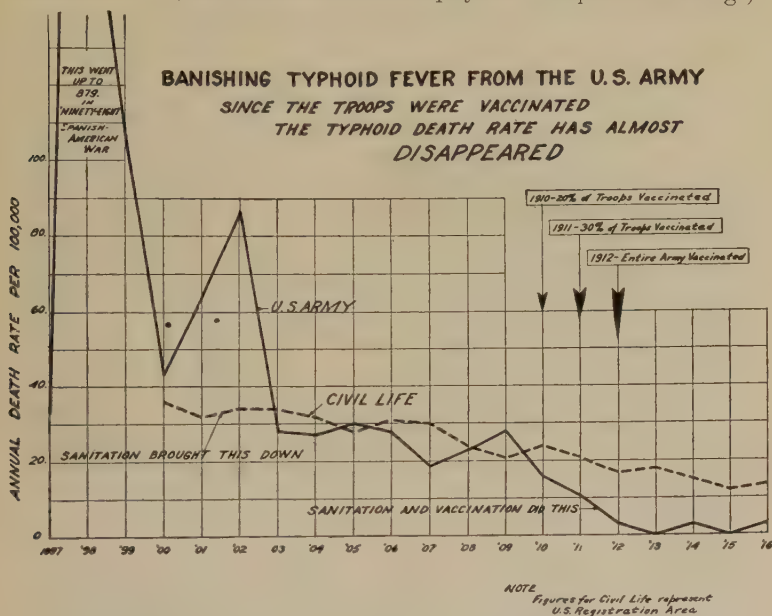


FIG. 128.—How do these rates compare with the rate for your state? VAUGHAN AND PALMER, *Journal of Laboratory and Clinical Medicine*, C. V. Mosby Co.

Recently Calmette has reported successful preventive work with tuberculosis vaccine, both with cattle and monkeys. An experimental station is now being constructed in West Africa to extend the observations of preventive tuberculosis vaccination among the man-like apes.

Objections to the Use of Vaccines.—While the ordinary subcutaneous vaccination is considered as without attendant risk, the intravenous mode may cause agglutination of the injected

organisms, resulting in obstructions in the smaller blood vessels; or, if the bacterial substances are too rapidly absorbed "shock" may follow. See the following chapter on Anaphylaxis for brief discussion of the "shock" and the dangers of repeated injections of bacterial proteins.

Other Phases of Vaccines.—The treatment given for hay fever, food sensitiveness, or any other form of "protein sensitiveness" is essentially the same as the vaccine treatment for bacterial infections. In any bacterial disease the stimulating substance given the patient is the specific bacterial protein,

while for food sensitiveness and hay fever patients, the related food or pollen protein is used. The description of the treatment in such cases is given in the following chapter, because of the close connection of the various phases of these problems with anaphylaxis.

While the substances used in the skin tests for the presence of diseases as tuberculosis, syphilis, and gonorrhea, are really the killed organisms, we do

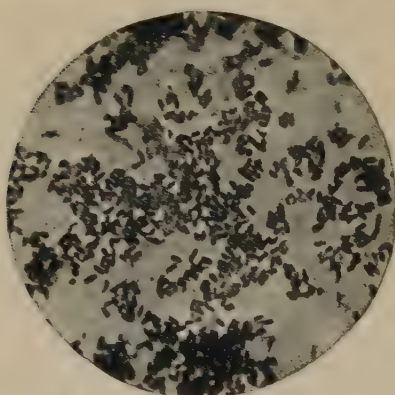


FIG. 129.—Plague bacilli, edge of mass of a culture from a flea's stomach (X 1000) MARTIN, *Journal of Hygiene*. (Eighth Report of Plague Investigation in India.)

not think of them as vaccines. True, the same bacterial substances may be used in both cases, but when used as vaccines they are given in sufficient amounts—whether in single or repeated doses—to excite an accumulation of helpful body reactions. When they are used to test for the existence of a given disease, but a single minute dose is given to aggravate and so make evident the sensitive condition of the individual. (See tests described on p. 205 and p. 208.)

STUDY SUGGESTIONS

1. Describe vaccine as first used and compare it with typhoid vaccine in origin (or preparation), dosage and results.
2. Why may we call the toxin-antitoxin treatment to prevent diphtheria a vaccine treatment?

3. List the vaccines advertised by a reputable firm; how many are bacterial and how many of protozoan origin?
4. What is an autogenous vaccine? What advantage may be claimed for autogenous over stock vaccines?
5. How may the strength of a vaccine be graded or measured?
6. What two vaccines do you consider most successful? Give supporting figures from army or national statistics.
7. Recently a large sum has been divided among three well-known institutions for the study of common colds. Prepare a list of organisms found in or causing diseases of the respiratory and adjacent membranes and show why it is improbable that a satisfactory preventive vaccine will result.
8. Killed cultures of *Leptospira icteroides* have been tried by Noguchi in 427 vaccinations with a morbidity of 11 per thousand among the vaccinated as compared with a morbidity of 110 per thousand among the unvaccinated. Have we in our country any other established vaccines for diseases of protozoan origin?
9. The statement is often made that curative results are more frequently obtained with vaccines in cases of localized rather than general infection. How may this be explained?
10. Many investigators recommend the employment of larger doses of vaccines than those given in the text of this chapter, e.g., 5,000 million to 25,000 million in gonorrheal vaccines. Can this be shown to be related to the chronic character of gonorrhea?
11. Karsner states that the "frequent occurrence of pneumococcus septicemia as a part of the disease makes it unlikely that vaccination will be helpful." Why?
12. In the city of Tokio 238,936 people were vaccinated against cholera. Cholera occurred in 1.85 per 10,000 of the unvaccinated people, and in 0.13 per 10,000 of the vaccinated. What was the relative morbidity of those not vaccinated?
13. How can you explain the good effects reported from the use of albumin solutions when used as a vaccine to prevent typhoid?
14. When measles was first introduced into the Faroe Islands, over 6,000 of the 7,782 inhabitants contracted the disease; why could measles not attain that percentage in any of our communities today?
15. Show what there is in common between diphtheria toxin-antitoxins and sensitized- or sero-vaccines.
16. Tabulate from an advanced text on bacteriology or immunity the diseases in which vaccines are used; separate or classify them as (1) live organisms, (2) dead organisms, (3) toxins.
17. The spread of smallpox has been limited so effectively by the "wall of the immune" people who have been vaccinated, that most communities are becoming very careless about enforcement of the smallpox vaccination regulations. How many of your community group (hospital, college, dormitory, etc.) would surely escape if an epidemic started?
18. Recently killed pneumococcus organisms have been used as a throat spray to arouse local reactions in the areas naturally first attacked in pneumonia infections. Give one argument in support of this method of vaccine administration.

CHAPTER X

ANAPHYLAXIS AND SIMILAR PHENOMENA

Anaphylaxis and similar reactions	{	Definition of anaphylaxis				
		Historical background	{	Experimental work		
				Underlying principles		
		Theories of anaphylaxis	{	Blood stream changes	{	Split-proteins
						Antibody-protein combinations
				Cellular theory		
		Time and quantity relationships				
		Anaphylactic manifestations	Experimental animals			
			Man			
		Anaphylaxis and protein sensitization				
Important human relations	{	Vaccines				
		Serums				
		Foods				
		Pollens				
		Tests for disease				
Anaphylaxis and immunity						

ALTHOUGH it is impossible to present the subject of anaphylaxis satisfactorily in one brief chapter of twenty pages, it seems desirable to bring together in this final chapter, various phenomena commonly discussed in connection with that topic.

Meaning of Anaphylaxis.—The term anaphylaxis (without or against protection) is used to cover a wide range of phenomena, varying from such slight and local symptoms as an itching of the nose and running of the eyes and nose to sudden collapse and death. (See p. 196.) More specifically it includes such widely various phases or occurrences as (1) true anaphylactic shock and death, (2) the less fatal "serum sickness," (3) the tempo-

rary discomfort following the second or later injections of vaccines, (4) such skin tests as those used for tuberculosis or gonorrhea, (5) the diet idiosyncrasies of certain individuals, and (6) the sensitiveness of other unfortunates to flower pollens or to epidermal cells of cats and horses.

Historical Background of Anaphylaxis.—About eighty years ago (1839) a French physiologist, Magendie, described the sudden death of dogs which had received repeated injections of egg albumen. In 1894, Flexner found that animals succumbed to the second dose of dog serum, if it was given several days or even several weeks after the first dose. The term anaphylaxis (without protection) for such susceptibility to proteins, was first applied by Richet, who with Hericourt, secured similar results with dogs following the second injection of eel serum, which, however, is itself a toxic substance.

With this came the dawning appreciation that (1) this phenomenon depended upon a previous inoculation with the same protein substance, and that (2) a definite time must elapse before such sensitiveness developed. In 1903, Arthus, a Frenchman, proved that this time interval was important, and that the phenomenon of anaphylaxis was not merely a matter of accumulating a given total amount of the foreign protein—for, while a single dose of 40 cubic centimetres of horse serum was not fatal, several repeated small doses of 5 cubic centimetres, or even less, caused anaphylactic disturbances.

Arthus also showed that the phenomenon of anaphylaxis was due to a general systemic rather than a local condition or sensitiveness, for the same anaphylactic results occurred even though the first and the later injections were in such entirely different areas as the skin and peritoneal cavity.

Much of this earlier work was, however, forgotten, or ignored through failure to grasp the essential likeness—the protein character of the several substances used in each of the experiments just described: egg albumen, horse serum, dog serum, and the (toxic) eel serum. Therefore, in 1904, when Theobald Smith accidentally found that his guinea pigs, which had been injected with horse serum antitoxin in some standardizing tests on diphtheria antitoxin, were very susceptible to plain horse serum when that was injected several days or several weeks later,

the anaphylactic condition that resulted became widely known as the "Theobald Smith Phenomenon." The greater prominence given Smith's results was due both to the increasing opportunities for exchange and diffusion of information concerning the achievements of the scientific world, and to the current wide interest in diphtheria antitoxin.

Since Smith's observation in 1904, many important contributions have been made to this problem. Very promptly followed those of Rosenau and Anderson (1906-08) who showed that antitoxin, *per se*, had no relation to the Theobald Smith Phenomenon,—for the same result occurred if plain horse serum were used in both injections in the experiment. This really reduces the Smith Phenomenon to a parallel of Arthus's results with horse serum and even to Magendie's egg albumen experiment back in 1839. Among other contributions of Rosenau and Anderson to the subject of anaphylaxis are the following: (1) The reaction is specific, that is, anaphylaxis is brought about by later injections of the same or specific substance (horse serum, egg albumen, etc.) used in the *first* injection. (2) Though this phenomenon is an extremely delicate one (for only one-millionth of a cubic centimetre may be sufficient to sensitize an animal), the susceptibility thus induced may persist for a long time, over three years being recorded in some test animals. (3) Anaphylaxis can be induced by many other substances, by vegetable proteins, including bacterial proteins, as well as animal proteins, such as those already described.

This last fact connects the problem of anaphylaxis with infectious diseases, and as later shown (p. 193), gives a different conception of the relation of the non-toxin-producing bacteria to disease. (See p. 22.)

Anaphylaxis a Protein Reaction.—Further explanation of the part played by proteins—not only in diseases, but in diet idiosyncrasies, etc., has been given by Vaughan and his co-workers. He has shown that widely different protein substances (white of egg, milk, peptone, seeds, non-pathogenic bacteria and also pathogenic bacteria) have the same fundamental basis of structure—a poisonous "core" or basic substance which is common to all protein substances, and may be obtained from any protein by appropriate treatment (with acid, alkali, alcohol,

etc.) which causes the breakage or splitting of the various kinds of protein molecules into smaller poisonous ones common to all proteins, the "poisonous split-proteins." With these split-proteins, obtained from sources as varied as those listed above, Vaughan has been able to demonstrate that by appropriate dosage any given type or set of clinical symptoms can be produced; these include various types of fevers (acute, relapsing) convulsions, and death, all of which may be produced in a given or predicted time.

The effects of repeated injections of proteins which have not been previously so disrupted or broken, as described earlier in the egg-albumen and serum experiments, can be explained in the light of these same poisonous split-proteins. When egg-albumen, for example, is injected into the tissues, some of it may find its way, relatively unchanged, into the blood stream; this is, of course, an unusual condition, for though egg substances are commonly used as food, they are then taken into the alimentary canal and there digested or broken down into simpler substances before passing into the blood itself. The blood does not ordinarily contain the mechanism (enzymes?) for taking care of the unchanged proteins; therefore appropriate substances must be developed and finally accumulated in the blood when the foreign proteins do find their way there. A first injection of any foreign protein may therefore stimulate the production of suitable protein-breaking substances which remain in the blood for some time (See p. 192). If during the period they persist in the body, we inject a second dose of that same kind of protein that stimulated them, it may be attacked at once by these special protein-breaking substances, and broken down very rapidly. Poisonous split-proteins produced by these processes, and accumulating in the blood, may be absorbed by the tissues more rapidly than they can be neutralized or further broken down to harmless stages, giving the condition known as anaphylaxis, or anaphylactic shock. The explanation is, of course, equally applicable to the anaphylactic manifestations occurring with other injected proteins—anti-serums and vaccines—or to any protein substances such as pollen and epidermal cells absorbed through surface membranes rather than definitely inserted or injected into the tissues.

Antibodies and Anaphylaxis.—The explanation of ana-

phylaxis just given, throws the emphasis upon the digestion products derived from the foreign protein. It is the simplest of several theories, and for that reason is given the most prominence in this elementary presentation.

Many find it, however, inadequate for some of the complicated protein relationships occurring in health and disease, and two other theories are often advanced to explain anaphylactic phenomena. According to one of these, the first injection of the foreign protein stimulates the production of suitable antibodies which combine with it; the second injection of that specific protein finds ready an abundance or superabundance of these antibodies with which it unites, forming thereby irritating or toxic products which induce anaphylaxis. Support is given this theory by workers who have shown for various bloods a definite relationship between the abundance of contained antibodies already familiar to us, precipitins, and the power of these various bloods to produce anaphylaxis when injected into other animals.

The Cellular Theory.—A third theory, which is rapidly growing in favor, differs from both of those just given, in locating the anaphylactic processes in the body cells—in the fixed tissues—rather than the blood stream. According to this theory, the first dose of protein sensitizes by inducing changes in the body cells—changes which make possible the combination of the foreign protein with the cells themselves. On the second dose of injection of the given protein, it makes ready combination with the body tissue cells, by virtue of the changes earlier induced. The formation of such antibodies as precipitins would fit into this third theory as well as into the second one, discussed in the preceding paragraph. Precipitins retained by the cells and not discharged into the blood stream would increase the cell sensitiveness and susceptibility to injury—through their increased combining power with the foreign proteins. The toxic condition according to this theory—the cellular theory—emanates from the body cells themselves.

To sum up briefly, we may consider the mechanism of anaphylactic states as due (1) to the formation of ferments or enzymes which digest or split the foreign proteins into harmful substances, or as due to the production of “antibodies,” such as precipitins, which injure the animal body by making antibody-

protein combinations—either (2) in the blood stream or (3) in conjunction with the body cells.

Quantitative Relationships: Time and Dosage.—In each of the three theories of anaphylaxis thus briefly delineated, we have a combination of a specific protein with a reacting substance produced in the body, and as one would naturally expect, the effect produced in any given instance varies more or less with the degree of combination taking place. If the second injection or dose of the specific protein is given before a certain necessary minimum of the reacting substances has been accumulated, anaphylactic results will not be produced. In man, a period of about 12 days is necessary for the production of such reacting substances. When repeated doses of the same proteins must be given, as in serum therapy and in most of the vaccine treatments, the repetitions are made short of this twelve-day interval; vaccines may be given at three-day or weekly intervals; and antiserums are usually repeated at very short intervals—such as every eight, twelve or twenty-four hours until improvement is assured.

Size of the Sensitizing Dose.—The amount of the foreign protein is also an important consideration—both with regard to the initial sensitizing dose, and the amount necessary to induce anaphylactic symptoms. Though a very minute amount may be sufficient to “sensitize” the animal (See p. 192), there is an irreducible minimum for even the first dose of the foreign protein; for some animals the sensitizing dose must be quite large—several cubic centimetres, and some animals, such as rabbits, require several repeated doses to really sensitize them.

Size of the Sensitizing Dose.—The amount of the foreign protein with regard to the amount which will cause the appearance of anaphylactic symptoms. With guinea pigs it may be but a minute amount—as small as $1/1000$ of a cubic centimetre. Two cubic centimetres is called a “shock injection” for a rabbit, and 20 to 30 c.c. will cause anaphylactic death in dogs. The amount of protein injected in the “shock dose” naturally limits the amount of toxic substances that can be produced, and the degree of anaphylaxis can be modified by suitable reductions in the protein given; the results may thus be made to vary from a slight local symptom, such as reddening or inflammation at the site of the injection, to general systemic disturbances, or collapse and

death. A very small amount of the protein will produce only local reactions, or slight increase in temperature, etc., indicating that the animal is sensitive to the protein in question. This is the basis of skin tests where minute amounts are used for determining whether or not an individual is sensitive to the respective proteins of such diseases as tuberculosis (See p. 208), or whether he will be affected by the usual dose of antitoxin. Not only can the degree of anaphylactic reaction be lessened (Fig. 138) by reducing the total amount of foreign protein given, but by spreading the full amount which would cause anaphylactic results, over a longer period—that is, giving it in divided doses at short intervals. This probably gives the tissues time to dispose of the poisonous products that result so that at no instant is a harmful total amount present in the body. Advantage is taken of this in giving antisera to over-sensitive individuals. In using diphtheria antitoxin, for example, instead of giving the whole dose at once, it may be given in very small subdivisions, but $\frac{1}{10}$ of a cubic centimetre at a time, at half-hour intervals, until the prescribed total has been injected.

Typical Anaphylaxis in Experimental Animals.—Recently much discussion has centred around the differences between these protein reactions as observed in man and in the lower animals used in experimental work. As observed in that very susceptible animal, the guinea pig, the anaphylactic condition may be described briefly as follows: A few minutes (or even as short a time as one-half minute) after the injection of the dose causing anaphylaxis, the animal shows a drop in temperature, restlessness and difficulty in respiration. The animal may then either recover slowly, or grow progressively worse, the general weakness increasing so that the animal falls upon its side, respiration becoming more difficult and slowed, the condition finally ending in convulsions and death. The whole process may be extremely rapid, death taking place within one minute after the “shock dose” is given.

The effects are attributed to the contracted condition induced in the smooth or involuntary muscle tissues, variations in the symptoms of the various laboratory animals corresponding somewhat to differences in the distribution and proportion of this sensitive muscle tissue in the affected areas, such as the lungs. In

rabbits, for example, the bronchial regions are less well supplied with smooth muscle, and the respiratory phases are less marked than in guinea pigs; similarly in dogs, the absence of respiratory symptoms, such as asphyxia, and the prominence of intestinal symptoms may be explained by the relative proportions of smooth muscle tissue in those respective areas.

Anaphylaxis in Man and Other Animals.—In man, symptoms of extreme severity are rarely produced. They occur but rarely even with serum treatment, in which large amounts of foreign proteins (See p. 79) are injected. According to Park, but one individual in every 10,000 treated with diphtheria antitoxin develops alarming symptoms, and but one in 50,000 dies. Most of the human disturbances fall under the less serious manifestations described in connection with serum sickness, food sensitiveness and hay fever, in the following pages.

Anaphylaxis as seen in the ordinary experimental animals, is a phenomenon affecting primarily the smooth muscle fibre, especially in the respiratory and circulatory areas; in man the manifestations are more varied, and the effects most often noted are in such areas as the skin or the nose and eye membranes.

What is apparently the greatest contrast is seen when we state that when fatal results do occur in man they follow quickly upon the *first* injection of the protein while anaphylaxis in experimental animals occurs after one or more preliminary sensitizing doses. Then, too, most of the effects occurring in man seem more dependent upon systemic peculiarities or inheritable predispositions. Individuals supersensitive to antitoxin, for example, are often found to have an individual or family history of asthma, food idiosyncrasies, etc. Anaphylactic conditions in guinea pigs and other laboratory animals have not yet been found to be associated with such inheritable characteristics.

The differences between man and the usual laboratory animals may be more a matter of dosage than is apparent. The rabbit has been described as being 400 times as resistant to induced anaphylaxis as the guinea pig, and man may have a similar high coefficient of resistance; it may well be that what is considered a difference between the human and animal type of such protein sensitiveness may sometimes be shown to be really a difference in degree. It is quite possible that the size of the final dose pro-

ducing the symptoms affects the quantitative or relative distribution of the poisonous substances to the various smooth muscle areas, and therefore affects the relative prominence of the respiratory or other regional symptoms.

Anaphylaxis, Allergy and Protein Sensitiveness.—Such differences as those listed in the preceding paragraph, have led some to limit the use of the term anaphylaxis to the acute symptoms most characteristically seen in the guinea pig, and to adopt for the various less fatal hypersensitive manifestations in man, another term, such as Von Pirquet's name, allergy (formed from *allos*, change, and *ergon*, action and indicating the changed or altered power of the animal to react). Others avoid making a sharp distinction of this kind, and either use (1) the term anaphylaxis in the same general way that we have so far followed in this discussion, or (2) substitute for it the more inclusive term, protein sensitiveness.*

Protein Sensitiveness.—The term protein sensitiveness has been recommended with the idea of avoiding too great emphasis upon differences in the final or outward physical manifestations, and emphasizing by the use of this inclusive term, underlying likeness in all such phenomena, the protein sensitive condition of the animal.

Unfortunately, this use of protein sensitiveness, leads to two other serious errors, although it does accomplish the immediate purpose and emphasizes the common fundamental likeness—that the various phenomena are all responses to foreign proteins to which the animal is sensitive. The first error involved is that such use of the term protein-sensitiveness implies the complete separation of these phenomena from all of the antibody reactions discussed in the previous chapters of this book, and leads the unwary to forget that agglutinins, opsonins, and all other antibodies are likewise formed in response to bacterial or other protein stimulation. The second error involved in this restricted use of the term protein sensitization is the implication that protein sensitization is essentially harmful and undesirable. This blinds the student to the fact that protein sensitization is essential

* In such a brief discussion many important phases must necessarily be omitted, *e.g.*, whether such sensitization really occurs with protein substances only, or whether such exceptions as those reported for lipoids are really attributable to protein-lipoid combinations.

to the development of active immunity, and that only certain incidental or attendant phases of such sensitization are harmful. To pick out these isolated and injurious exceptions and call them protein sensitization is certainly misleading. It is highly desirable that we should refrain from increasing in the slightest degree any objections the uninformed may still hold against such invaluable preventive methods as those involving the use of vaccines and antisera.

Drug Sensitiveness.—Under the term allergy, is sometimes included the supersensitiveness of certain individuals to such drugs as iodine, quinine, morphine, mercury, turpentine and salvarsan; the reactions may be very similar to those observed in human hypersensitiveness to proteins, with such symptoms as fever, local inflammations, swollen joints and lymph nodes, and skin eruptions. With drugs, as in the human reactions against pollens and food proteins, a high degree of immunity is not developed—the reaction following repeated treatment or vaccination being tolerance rather than true immunity (See p. 207).

Anaphylactic Considerations in Relation to Vaccines.—Since bacteria are protein substances, their continued repeated injection might be expected to cause anaphylactic reactions. The increased temperature, local inflammation, etc., attending the later injections of a given vaccine, such as typhoid vaccine, is due in part, of course, to the actual increase in total protein, but in greater part to the higher sensitive or anaphylactic condition of the individual. Vaccination has, therefore, been described as “extended or continued anaphylaxis.”

Students often ask why vaccines can be given throughout a long period of time without risk of causing serious phases of anaphylaxis. Even though the second or even the third dose may safely fall within the twelve-day period (See p. 195), why do not the later injections cause dangerous anaphylactic symptoms? After any given dose or injection there is a short period—several days or even weeks—during which the individual is less susceptible to anaphylaxis—during which the ordinary anaphylactic dose will not affect the animal. These less sensitive or refractory periods are similarly extended throughout the treatment, and make possible, therefore, continued injection of vaccines, which

may be extended through weeks or even months, as in staphylococcus treatment.

There is another possible explanation. The first dose of a given vaccine may not have been sufficient for a sensitizing dose; it may even require—for that individual or for the strength of the vaccine used—more than the usual number of injections to sensitize that individual. (See p. 195.)

Vaccines, therefore, are usually given without serious discomfort and without risk. The discomfort following vaccine injections is usually greatest after the second dose, and is usually limited to soreness in the locality of the injection, pain in the adjacent glands, and general indisposition, sometimes accompanied with headache or nausea.

While vaccine treatment is quite generally approved for typhoid, dysentery, smallpox, cholera and also staphylococcus infections, many feel that it is less warranted in tuberculosis,—both because of the greater length of the period during which the vaccine (tuberculin) injections must be continued and because of the more damaging effects of the tuberculosis proteins. Since clinical symptoms do not always warn us of the erratic changes occurring in tubercular people, injections may be given at a very inopportune time, when the body is not able to counterbalance the effects of the organisms growing in the tissues, and may thus add unnecessarily to the work to be done without giving the desired stimulus or aid. While tuberculin treatment may “promote healing and make relapses less frequent,” it may hasten or increase the conditions it is intended to overcome, and experienced physicians recognize that “such a weapon is a two-edged sword.” This objection does not, of course, apply to the use of tuberculin as a test for tuberculosis (See p. 208), where we use but a minute amount in a single injection.

Serum Reactions in Man.—As seen in human beings there are two important types of serum reactions. In the most serious type the patient promptly (five to ten minutes or even a shorter time after the first injection) experiences collapse, unconsciousness, and great difficulty in breathing. Less than half of such collapses result fatally, death occurring, as noted elsewhere (p. 103), in but one case in every 50,000 diphtheria patients treated with antitoxin.

Serious results are more common with patients who are asthmatics, horse-sensitives, etc. (See p. 197). Before injecting any antiserum the physicians should ascertain whether the family or individual history indicates such hypersensitiveness, and in such cases the risk should be clearly explained, and the dose given with appropriate precautions. Among such precautions are the following: (1) giving first a trial dose of a very small fraction of a cubic centimetre, for even a single cubic centimetre has proven fatal in such cases (See p. 200); (2) giving the dose very slowly either spacing it throughout several hours, (See p. 196), or giving it in oil combinations to insure its slow absorption; and (3) having at hand appropriate drugs (chloral hydrate, atropin, adrenalin) for lessening the anaphylactic effects on respiratory or other smooth muscle tissues (See p. 196).

Serum Sickness.—The more common after results, but fortunately not fatal ones, are the several effects grouped together under the name "serum sickness." These phenomena may appear in a few hours, but more often between the third and twelfth day following the injection of the antiserum. They include various types of skin eruptions, excretion of albumin in the urine, swollen lymph glands, especially near the injection site, and painful and sensitive joints. The frequency with which such symptoms follow treatment depends upon such variables as the size of the dose or the amount of foreign protein given and the reduction or elimination of the serum proteins.

Fortunately, the most frequently given antiserum, diphtheria antitoxin, is so modified in its preparation (See p. 93) that it has a minimum of protein material, and serious after-effects are rare. Every effort should be made to insure the general recognition of the rarity of serious results accompanying antiserum treatments, especially in diphtheria, the disease in which antiserum is most commonly used.

Reactions with Initial Rather Than Repeated Doses.—Little fear need, therefore, be felt in repeating serum injections during any attack, for the doses usually fall well within the initial twelve-day period of safety, and when this is not the case within the extended refractory period as described earlier on page 199.

As stated above, the serious results attending the use of anti-

serums occur with the first injection; as commonly expressed, "There is no danger from the second or later injections if the first was given with safety." The preceding paragraph has called attention to the fact that the time is too short for the first dose to act as a sensitizing dose. What puzzles students is why there should be any reaction with the *first* dose.

Among the theories extended to meet this situation are the following: (1) The irregular absorption of such first injection, due to the striking of a small vein, as might occur in a subcutaneous injection; the first absorbed fraction of the dose might act as the sensitizing or first dose, for the more slowly absorbed remainder of the dose. Or the injected substance may be virtually a second dose, the reaction being due to either of the two following causes: (2) Natural sensitiveness, through substances acquired before birth, *e.g.*, from the mother's blood. (3) Earlier absorption of similar substances through the alimentary or respiratory membranes—of substances enough like some of the injected proteins to cause typical anaphylaxis. This, of course, it is impossible to prove, but the plausibility of something of this kind appears when we recall that serious results, as already mentioned, are more common with people who exhibit other phases of protein sensitiveness. Some cross-relationship of this kind is also indicated by the fact that individuals injected with egg albumen to cure an egg-sensitive condition, may find that horse-sensitiveness or other idiosyncrasy disappears with the egg-sensitiveness.

Anaphylactic Phases of Serum Treatment.—Antiserums may be administered in but single doses; this is commonly true of diphtheria antitoxin as used in curative work and in the ordinary preventive treatment for tetanus. Where possible, but one dose of antiserum is given as in diphtheria treatment; the first dose is usually large enough to make repetition unnecessary. In many cases, however, more than one dose is necessary; three to five doses are often administered in the serum treatment of pneumonia. In the treatment of soil-infected or otherwise evidently contaminated wounds in the recent war, three sets of tetanus antitoxin injections were given around the outer edge of the wound as a matter of routine, one at the time of the first dressing, one early in the second week, and one during the third

week. This repetition was necessary because tetanus antitoxin is apparently eliminated from the body in eight to ten days.

Where repeated injections of antiserum are used in other diseases they are usually repeated at even shorter intervals: every twelve or twenty-four hours in lobar pneumonia, and every eighteen to twenty-four hours in meningitis.

Food Sensitives.—There seems little reason to doubt that unusual forms of protein may make their way into the blood stream from the alimentary canal. Such absorption seems to take place more readily from the large intestine than from other parts of the alimentary canal, and in digestive disturbances, incompletely digested or unchanged proteins may reach the large intestine. Severe operative shock and the attending lack of tone in the intestinal region apparently favors such absorption of incompletely digested proteins.

It has been claimed that parents, by giving to a child too large an amount at the first feeding of a new food, such as egg, may create a sensitiveness to that food, by inducing an overproduction of the reacting substances which persist and cause disturbances later when that food is eaten. More often, probably, there is a family history of asthma, hay fever or similar food sensitiveness that may be a more important consideration (See p. 201). Such sensitiveness may appear most erratically, however, and without any known family or inherited sensitiveness; for example, in one individual a sensitiveness to strawberries occurred for the first time at the age of fifty and lasted throughout an entire season. Such sensitiveness to foods is more common for milk and eggs than for any other foods. It may evidence itself merely by a hive-like or itching rash, or as an eczema, or it may be attended by most severe symptoms including respiratory difficulties and intestinal disorders. It is often inconvenient and sometimes quite undesirable to omit entirely from the diet the protein irritating to the individual. This is especially true for milk and eggs. While with time, improvement may occur without definite treatment, treatment is often necessary for full or permanent relief. This is gained in many cases by giving the individual gradually increasing doses of the specific protein. Since in food sensitives (Pl. I) the absorption seems to be by way of the alimentary canal, treatment is most simply accomplished

by giving gradually increasing doses of the specific protein by mouth—often in gelatin capsules. The initial dose may be as small as 1/1000 of a gram, and a few months may suffice to cover the entire period of treatment.

Sensitization Through the Respiratory Membranes.—

Absorption of foreign protein may occur through the nasal and lung membranes as well as through the intestinal membranes. Small and easily-floated particles of foreign protein substances, such as pollen grains, particles of goose and chicken feathers and epidermal cells (hair, dandruff) of horses, cats, dogs, and guinea-pigs, are among the irritating substances shown to be absorbed in this way. Most of us know of people who cannot work with horses or guinea pigs, or who have asthmatic symptoms or hive-like outbreaks on the skin if they drive behind a horse even a short distance. It is only recently that we have understood the peculiar phenomenon of people who are made uncomfortable by the presence of cats—who “know without seeing when a cat has entered the room.”

More common are cases of discomfort due to the absorption of plant proteins, such as flower pollens. Ragweed, grasses, such as timothy or the staple grains, early flowering trees with abundant wind-blown pollen and several plants with more conspicuous flowers, such as daisies and roses, are common causes of “hay fever,” “rose cold” or similar respiratory difficulties. Goldenrod is probably less important in this connection than generally supposed. Very fine particles of other plant parts such as dust from broken grass seed or wheat grains may have the same effect upon similarly hypersensitive people. (See p. 213.)

In all these cases the sensitive condition thus provoked is no doubt due to earlier and unnoticed absorption of the substance the individual finds so irritating. On each later opportunity enough is absorbed to produce anaphylactic symptoms. The extreme susceptibility of some individuals is attributed to a difference in the mucous membranes of the respiratory area—to the “peculiarly acute penetrability” of such membranes. There is also evidence that these differences vary in a given individual with his physical condition (disease, fatigue, operative shock, etc.). This would explain many of the erratic manifestations,

such as the sudden susceptibility of a hitherto immune individual, or recovery without treatment.

The amount of substance which causes these anaphylactic symptoms or conditions is usually very slight. Vaughan reports one individual who on entering the laboratory could tell by the presence or absence of disturbing symptoms whether or not the cork was out of the peptone bottle.

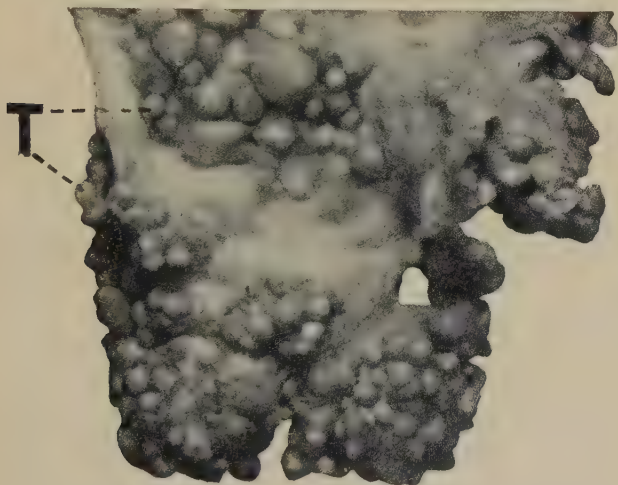


FIG. 130.—This photograph with numerous tubercles (T) on the omentum or fat layer shows the reason for the name tuberculosis. (External view) MOORE, Bovine Tuberculosis. Macmillan.

Tests to Identify the Specific Causes of "Hay Fever" and Food Sensitiveness.—In all conditions of food sensitiveness and sensitization through respiratory membranes, the first thing required is to ascertain definitely what protein is responsible for the anaphylactic symptoms. This is ascertained by means of intradermal skin tests. Minute quantities of different proteins are introduced beneath the outer skin. (Fig 137.)

Eight or ten different substances are often injected at the same time, one below the other, an inch or so apart, in the upper arm or the leg. The relative degree of inflammation at the respective injection sites, enables the physician to decide which is the most irritating substance for that individual. (Fig. 138.)

Treatment for "Hay Fever."—Occasionally an individual is able to control his time to such an extent that he can avoid the irritating cause, *e.g.*, avoiding ragweed pollen by spending the summer in the city, mountains or summer resorts where that particular plant does not grow. Often he prefers to "take treat-



FIG. 131.—This shows a cross section through the lung tissue and so through many of the tubercles. MOORE, Bovine Tuberculosis, Macmillan.

ment," the treatment consisting of a series of appropriately graduated doses of the protein to which he is sensitive; this makes him finally less sensitive, probably because the body tissues have learned to take care of that particular protein in a different way—either gaining the power to break it down without an accumulation of the poisonous split-proteins, or forming substances to protect the tissues against these poisonous split-proteins.

Treatment may not give the patient complete or permanent

freedom from his difficulties, as he gains tolerance rather than complete immunity, apparently. For treatment, an extract of the specific irritating substance is used. Pollen, for example, may be prepared by drying the pollen from the plant in question,

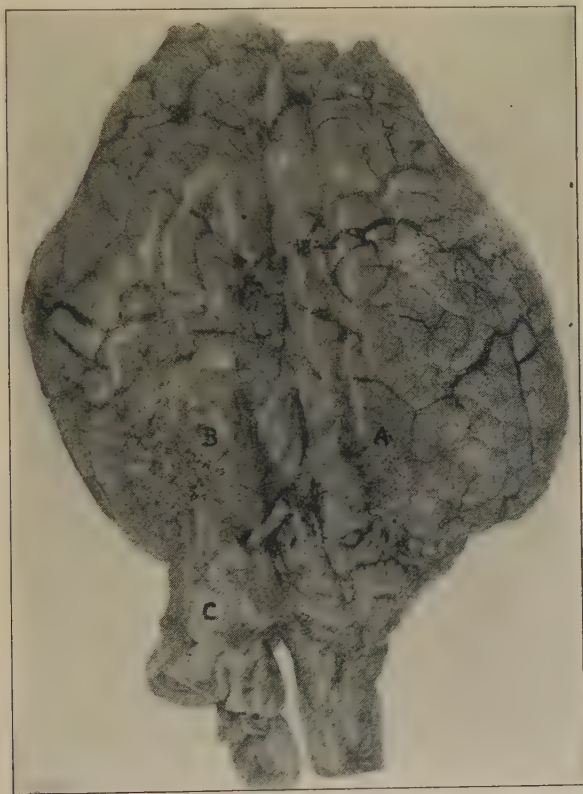


FIG. 132.—Lesions (A, B) in brain of cow, due to tuberculosis organisms. MOORE, Report New York State Veterinary College.

and then treating it with a glycerol and salt solution to secure the extract needed.

Tests for Disease.—As already stated (p. 196), bacterial proteins, because of their anaphylactic relationships, may be used in testing for disease. These tests may be used to show either (1) the actual presence or absence of a given disease in the

tested individual, or (2) the immunity or susceptibility of an individual to a given disease.

The diseases of most interest in this connection are tuberculosis, syphilis (Pl. I) and gonorrhea. Since the test for tuberculosis is the one most generally known, that may be described as more or less illustrative. The principles underlying such tests do not differ from those applying to hay fever and food-sensitive tests (p. 205).

The (Von Pirquet) Tuberculin Test.—The various preparations of tuberculosis organisms used in the tuberculosis

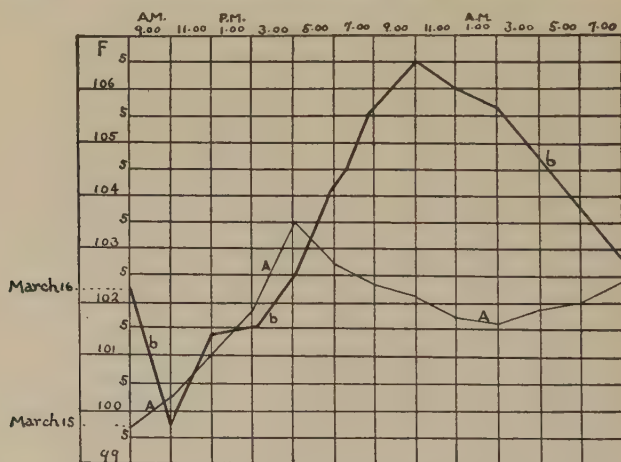


Fig. 133.—The light line shows the normal temperature of a cow; the heavy line shows the greater range in temperature (positive test) obtained with the same cow the next day following the injection of tuberculin. Redrawn from MOORE, Pathology of Infectious Diseases, Macmillan.

test are called tuberculin. Many different types of tuberculin are prepared in the various laboratories—heated and unheated, concentrated and not concentrated, broth cultures and emulsions, with and without glycerine, etc.

The various types are indicated by special names, abbreviations, etc., such as Tuberculin, B. F., the final initials in this case indicating a bouillon filtrate.

The tuberculin test is made by injecting some of the tuberculin into the individual to be tested, usually into the skin. Several methods are used: such as scarifications in the skin (cutaneous method), or, inserting the tuberculin into the sub-

cutaneous tissue (subcutaneous method) ; in the newest and most commonly used (intra-cutaneous) method, the material is inserted between the layers of the skin just as in the Schick test. Injections in the eye, a method often used with cattle, is rarely used in man.

In a tubercular individual the characteristic skin response to tuberculin is a reddening and swelling at the site of injection, beginning in about six hours, increasing through the first

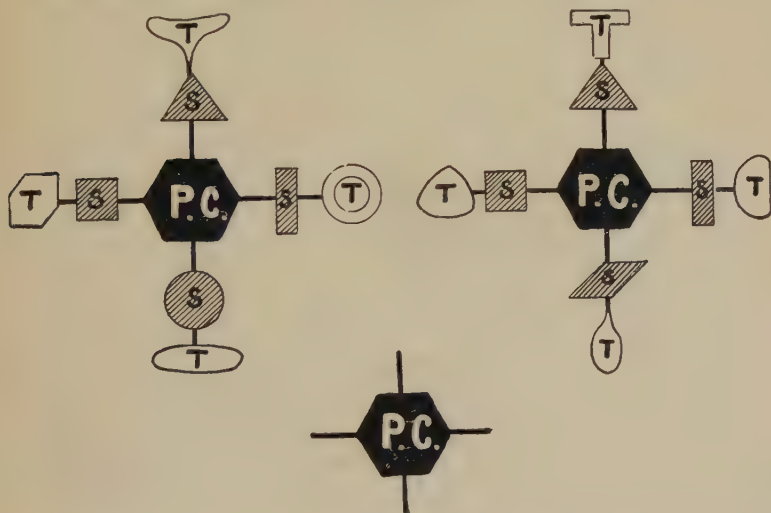


FIG. 134.—A diagrammatic representation of two proteins to show the very diverse character due to different secondary (S) or tertiary (T) parts. It is possible to split each down to the same poisonous core. (P.C.) See also Fig. 12.

two days following the injection, and disappearing in six to ten days. People who have not tuberculosis, whose tissues are not already irritated by tuberculosis organisms, are not affected by such small amounts of tubercle bacteria, and do not, therefore, show such local irritation. (See also temperature reaction, Fig. 133.)

Positive reactions are obtained only from people who have tuberculosis or who have recovered from tuberculosis. On the other hand, a tubercular individual may not give a positive reaction the first time, a second injection being necessary to sufficiently stimulate such response. Occasionally advanced cases fail to give a positive reaction ; in such cases, however, the clinical

or physical symptoms are usually sufficiently indicative. The principal argument against the tuberculin test, however, is that a positive reaction may be obtained not only from latent but also from cured cases, and that, therefore, such reaction is not very helpful for adults, since practically all adults have at some time had tuberculosis—mainly mild or unnoticed cases. (Sputum examinations are, of course, determinative in pulmonary types of tuberculosis, and may be used to support the tuberculin tests.)

Other diseases, such as typhoid and syphilis, lessen the diagnostic value of the results obtained by tuberculin tests. (See also agglutination and complement-fixation tests for tuberculosis.)

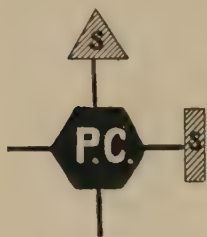


FIG. 135.—Even if the splitting is not so complete, a like body may be obtained as shown in the following figure.

For young children, tuberculin tests are more decisive and may throw light upon otherwise perplexing conditions, such as sometimes exist with gland or intestinal tuberculosis; in a young child a negative tuberculin test would eliminate tuberculosis and indicate the cause should be sought elsewhere.

Skin reactions similar to those described for tuberculosis, occur with emulsions of *Treponema pallidum* in skin tests for syphilis. Skin tests are also made with typhoid and gonococcus organisms in testing for typhoid or for gonorrhea. These tests are not nearly so reliable as the tuberculin test—as non-specific reactions may occur with “relative frequency,” and many authorities consider these tests far from satisfactorily verified or established as yet. Illustrations of characteristic reactions in positive skin tests, as in gonorrhea and syphilis, may be found in Plate I.

Symptoms in Disease.—Since one of the theories of the poisonous effects of bacteria implies a common poisonous substance (See pp. 20 and 192) two questions often arise. One is, “Why then do different diseases have different symptoms?” or even, “Why are there different diseases at all?” The second is, “How can we then test for a given disease; are such tests really specific and reliable?”

In answer to the first we may say that although all proteins *can* by laboratory processes be broken down to a similar form, it does not at all follow that they are all so broken (Fig. 135) in

the body, and in the stages actually reached we may find similarity without complete likeness. (See Fig. 136; also Fig. 13.) Vaughan explains the different symptoms in the various diseases by attributing to different bacterial by-products dissimilar chemical affinities which cause them to accumulate in different tissues of the body, *e.g.*, lungs, kidneys. These varying "predilection centres" give us the various symptoms or combination of symptoms associated with the various diseases.

In answering the second question, "Are such tests really specific and therefore reliable?" the results compiled show that, as described in discussing the tuberculin test, positive results are truly indicative of present or past infection. Negative results, here as in every other field of science, are not positive evidence.

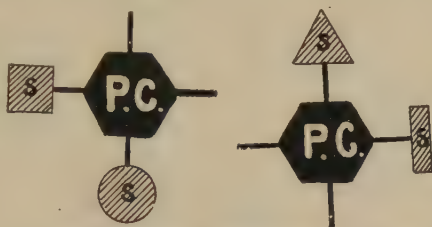


FIG. 136.—Even though proteins can be split into the like poisonous substances, proteins probably differ with regard to the ease with which this may be done. The formation of such incomplete split products, differing slightly from each other, as shown here, may explain the different effects and symptoms when different bacteria (proteins) invade the body. See also Fig. 13.

Anaphylaxis and Immunity.—The sensitiveness which produces reactions against bacterial proteins can not be separated from the sensitiveness which initiates the production of helpful antibodies against them—so alike are the conditions known as anaphylaxis and as immunity. The changes upon which the development of immunity depend have their attendant dangers; in the anaphylactic disturbances we see "the disadvantages of the advantages" hoped for in immunity.

This chapter forms but a brief presentation of the varied and perplexing phenomena ordinarily discussed in connection with anaphylaxis—the most hotly disputed subject in the whole realm of immunity. Nowhere do we find agreement or unity—with regard to the theory, the terminology, or the manifestations of anaphylaxis. In this discussion the aim has been to maintain a safe middle course through this perilous region, hoping that the beginning student will find herein what seems to him a simple, sensible explanation of the situations in his past experience, and, also what will prove, later, an easy introduction to a real study of this most interesting if complex subject.

STUDY SUGGESTIONS

1. Show that anaphylaxis is a protein sensitiveness.
2. What is the "Theobald Smith Phenomenon?"
3. What is serum sickness?
4. Describe anaphylaxis in an animal other than man.
5. What human tests to show the presence of, or the susceptibility to, disease are based on anaphylactic reactions?
6. The following is taken from a set of laboratory directions for procuring serum lysins against rabbit's red blood cells:
Inoculate a rabbit with sheep's cells.
 - (1) 1 c.c. 50% cells on first day, (2) 1 c.c. 50% cells on second day,
 - (3) 2 c.c. 50% cells on third day, (4) After 5 days repeat series,
 - (5) After next 5 days draw 5 c.c. blood from ear of rabbit.
 Through how long a period is the rabbit inoculated with foreign cells? Why does anaphylaxis not occur in rabbits so treated?

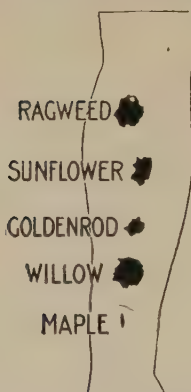


Fig. 137—Skin tests to determine the individual's susceptibility to several different pollens; reading down, these are ragweed, sunflower, goldenrod, willow and maple. Maple is used as a control since the usual results are negative. This individual should receive treatment with ragweed and willow pollen. Courtesy of Arlington Chemical Company.

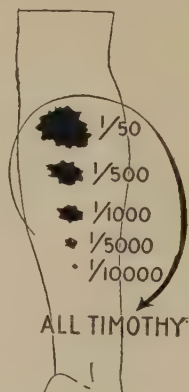


Fig. 138—Tests with varying strengths of timothy pollen to which this individual is sensitive to determine the strength with which treatment should begin, 1 to 10,000 in this case. Courtesy of Arlington Chemical Company.

7. How do we explain the different skin reactions obtained in Fig. 137?
8. In an early discussion of the antibody-extracts, (p. 71), their relative freedom from irritating results common to foreign proteins is supported by the statement that "even 5 c.c. of the extract only irregularly sensitizes." Show that this does support the claim made.
9. During the training of one of our men in the recent war he was shifted several times from camp to camp, and his records being lost or delayed, was repeatedly vaccinated with the (triple) typhoid vaccine, receiving in all nine injections in forty days. What is the probable explanation of the intense local reaction he experienced in the latest injections? Explain the lack of general anaphylactic symptoms during this whole period.

10. The intestinal wall of new-born infants (4th to 10th day) has been shown to be very permeable to small quantities of white of egg. What disadvantages may result from too early feeding or even giving a small "taste" of such proteins to young babies?
11. Explain or support the statement often made that for adults only negative tuberculin tests are to be relied on, and those only when no general malnutrition exists.
12. A recent article on "hay fever" cites the following list of causes: Pollen of birch, maple, roses, goldenrod, asters, daisy, clover, corn, timothy, ragweed, alfalfa; epidermal cells (dandruff, hairs) of horses, cats, dogs, rabbits, chicken feathers, cotton; insecticide powders; powdered orris root (in scented talcum powders); and broken particles of wheat, straw and cereals. Check off those found to be irritants by your acquaintances. Which cause is found most frequently? Which is not found at all?
13. *Health News* (October 1921) gives the following series of positive reactions in 100 consecutive asthmatic patients:

	Per cent.		Per cent.
Ragweed	28	Straw	12
Dust	25	Rabbit epithelium	8
Orris	19	Dog epithelium	7
Chicken epithelium ...	19	Dog saliva	6
Hay	19	Duck epithelium	3
Cow epithelium	16	Goose epithelium.....	3
Horse epithelium	14	Horse serum	3
Cat epithelium	13	Cotton	2
Timothy	13	Le Page (glue)	1

(Note, as the total of these percents indicate, that some of these patients were sensitive to more than one substance.)

Among your affected acquaintances do these ratios hold? How many of them are also sensitive to such foods as egg, milk, fish, cereals (wheat, corn and rice)?

14. Investigators have found that supersensitiveness to proteins as seen in man may be inherited (following Mendel's law of inherited characters). Does this preclude the explanation of such sensitiveness as being due (1) to the chemical sensitiveness of the individual's cells, or (2) to the unusual permeability of the exposed or contact membrane (nasal membranes, intestinal linings)?
15. Animals repeatedly injected with small doses of proteins may establish such a high degree of resistance that they can withstand 1,000 times the dose usually fatal to that animal. Is this high or low compared with the immunity following the use of *bacterial* vaccines, such as given in Fig. 119?
16. Use Ferry's characterization that anaphylaxis is "a phenomenon rarely seen in the human subject and seldom observed in any animal other than the guinea-pig"—a "picture that belongs to the research laboratory and not to the bedside," as a theme for dispelling the fears of the public with regard to the use of vaccines and serums.
17. One investigator succeeded in sensitizing a guinea-pig by such a small dose as 0.000,000,05 gm. of crystallized egg albumin. Can you find a record of a smaller sensitizing dose than that for any animal?
18. One firm advertises "No mixed pollen solutions for either diagnosis or treatment will be supplied." Do you approve of this? Why?

GLOSSARY

While it is presupposed that the reader has had an elementary course in bacteriology, the scope of the terms herein defined has been extended considerably to aid in making the book understandable to others who may be interested in the subject of immunity.

These definitions are, in no sense, complete technical definitions, but are designed as brief characterizations to aid beginners in using this text. Reference to the index will, in many cases, indicate the various passages in the main body of this book where these terms are more fully defined.

For information regarding the scientific name of a given organism consult the list following the glossary.

Acne. A skin disease, commonly affecting the sebaceous glands of the face, and characterized by such symptoms as dilated blood vessels and pustular or other types of eruptions.

Agar. The term agar is used for a semi-transparent substance resembling gelatin in appearance which may be expressed from seaweed, and also for a combination of that substance with meat broth to form a solid medium suitable for cultivating bacteria.

Agar Plate. A covered glass dish (Petri Dish) containing a layer of agar which furnishes food material for the growth of bacteria.

Agglutinins. Substances accumulating in the blood, in response to the specific irritation or stimulation caused by many foreign cells, such as typhoid bacteria. These substances cause the clumping together (agglutination) of the related bacteria—a phenomenon aiding in their

destruction. The name, agglutinins, refers to the apparently sticky or glutinous condition of the cells.

Aggressins. (Aggressive or attacking substances.) Enzymes or other substances formed by bacteria which increase their effect upon the host.

Alexin. Same as complement.

Allergy. (Working in another way.) The altered power of reaction occurring in animals as the result of inoculating with a specific substance; a type of protein sensitiveness. (See index.)

Alveolar. Relating or belonging to the alveoli, the air sacs or pouches which form the tiny ultimate subdivisions of the lungs. Although the alveolar membranes forming these sacs are richly supplied with blood, bacterial invasion may take place through these membranes.

Amboceptor. See "immune body." (Literally, holding two at once.)

A term used for the "immune body" and indicating its power to unite with two things at once—with another complementary blood substance (complement) and the irritating factor, (bacteria or other foreign cells).

Anaphylaxis. (Without protection.) A term covering various forms or manifestations of unusual or extreme susceptibility to foreign proteins; certain untoward phases of protein hypersensitiveness.

Antibodies. Protective reacting substances formed in response to such irritating factors as bacteria or their products. The names given antibodies—antitoxins, agglutinins, opsonins, and lysins—are more or less indicative of their action. More than one kind of antibody may be aroused by a single kind of bacterium.

Antigen. Any substance which when present or injected into an animal can stimulate the production of (one or more kinds of) antibodies. A vaccine is an antigen; any substance used to arouse sensitiveness (and therefore reacting antibodies) is an antigen. In blood tests, such as the Wassermann test, the term antigen is used for any substance capable of uniting with a given antibody.

Antiseptic. Against or preventing sepsis (or decay). Gauze used for dressings is sometimes made antiseptic by impregnating it with chemicals which inhibit or

prevent the development of bacteria in the wounded areas; usually dressings are merely sterile or aseptic. (See Sterile and Aseptic.)

Antitoxins. The specific substances or antibodies formed in the body in response to the irritating or poisonous toxins of such bacteria as diphtheria and lockjaw are called antitoxins. These antitoxins neutralize the toxins formed by the related bacteria, but do not destroy the bacteria themselves.

Popularly, antitoxin is often used to designate commercial diphtheria antitoxin, as produced by the horse, ignoring the fact that in such a disease the human body may itself produce its own antitoxins.

Arthritis. A disease of the joints with symptoms resembling rheumatism or gout. Various species of bacteria, e.g., *Streptococcus viridans*, are sometimes found to be associated with such conditions.

Aseptic. Without sepsis, putrefaction or decay. Dressings and instruments may be made sterile or aseptic by heat. In the effort to keep wounds in an aseptic condition they are, if possible, made surgically clean (removal of pus, diseased surfaces), and then either sterile or antiseptic dressings are used. See Antiseptic.

Auto-intoxication. The digestion or breaking down of protein

foods includes many temporary or transitional stages, some of which are poisonous. Nervous or other functional derangements of the digestive processes may lead to the accumulation and absorption of unusual amounts of some of these intermediate poisonous stages; this "self-poisoning" is termed auto-intoxication. The kinds of bacteria present in the alimentary canal vary in health and disease; and the prominence of one or more undesirable types may be responsible for the development of such cases of auto-intoxication.

Autopsy. An examination of a dead body to determine the cause of death, the seat of disease, etc.; a post-mortem examination.

Bacillus. (pl. *Bacilli*.) Rod-shaped bacteria; a term used usually for the rod forms possessing motility or the power to form spores.

Bactericidal. Able to kill bacteria; carbolic acid, alcohol, etc. are bactericidal substances. A given blood may have a bactericidal effect upon bacteria through the action of the white corpuscles, or the presence of such antibodies as lysins.

Bacteriemia. Bacterial infection in which the bacteria escape from the more or less localized lesions and invade the blood stream.

Bacterin. Bacterin is a term sometimes used as a name for killed cultures of bacteria which are used to produce immunity to a given disease. The vaccine

used to prevent typhoid is technically a bacterin. (See vaccine.)

Bacteriolysin. A lysin or dissolving substance formed against bacteria. (see Lysin.)

Bacteriophages. See white corpuscles.

Bacteriotropins. See Tropins.

Blood. See Serum; also Whole Blood.

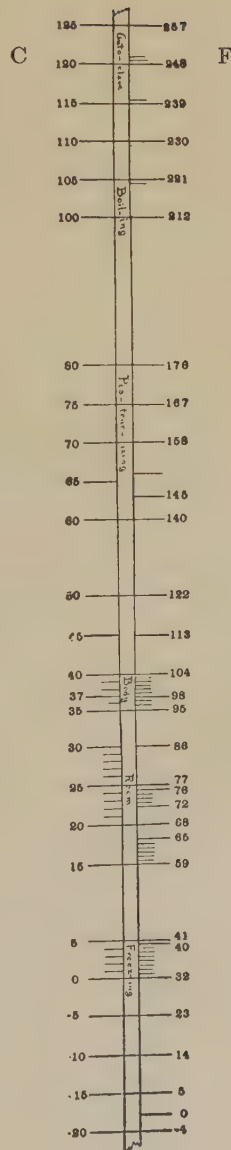
Blood Count. A count of the total number and proportion of white and red corpuscles. This count is made by putting a carefully measured amount of accurately diluted blood on a special slide which holds a given amount of blood over a central area marked off in tiny squares to facilitate counting. See index for illustration page. The count is made through a microscope.

Botulism. Food poisoning due to the accumulated toxin of *Clostridium botulinum*. Although several recent workers have obtained vigorous growth at body temperature, it still seems probable that few if any cases of botulism come from the development of the organism in the human body. Nevertheless canned or prepared food of a suspicious character (odor, texture, etc.) should not be eaten even if brought to the boiling point (or 15 minutes at 90°C.) which destroys the toxin accumulated in the food. The temperature necessary to kill the spores varies with their age, one

month old spores being "three times as resistant as five months spores." Botulism occurs most often after eating foods incompletely cooked (canned fruits or vegetables) or in which the preparatory processes (chopping pickling, ripening, etc.) cover extended periods as with some meat products, "ripe olives," etc., because the initial preserving conditions have not killed the organism or the later conditions have allowed its entrance into the food.

Carrier. A person, who though in apparent health, carries the organisms of a given disease in his body: diphtheria bacteria on the throat membranes, typhoid bacilli in the intestinal area, or malaria organisms in the blood. An individual may be a temporary carrier following the period of convalescence. It is estimated that 1 to 5% of typhoid patients become more or less permanent carriers; records have shown the persistence of such conditions for forty years and more. Less often, normal individuals, not known to have had a given disease may be found to be carriers of the organisms causing that disease.

Centigrade. (*centum*, one hundred.) The Centigrade thermometer has between freezing and boiling but one hundred divisions or grades, therefore, literally centigrade. The cut following gives the corresponding Centigrade and Fahrenheit tempera-



tures with rules for translating one scale into the other.

To convert degrees Centigrade into degrees Fahrenheit: multiply by 9, divide by 5, add 32.

To convert degrees Fahrenheit into degrees Centigrade: subtract 32, multiply by 5, divide by 9.

Centigrade:

C.C. (See Cubic Centimetre.)

Clinical. This term refers to the immediate or direct examination of a patient; the clinical symptoms are those which might be determined by observation at the bedside rather than by the microscopic or chemical findings of the laboratory.

CO₂. Carbon dioxide.

Coccus. (pl. *Cocci*.) Globular bacteria are termed cocci. The various bacteria of this general character are further differentiated or classified by other morphological or by functional characteristics, such as arrangement in chains (*Streptococci*), arrangement in packets (*Sarcina*), forming red pigment (*Rhodococcus*).

Communicable. Diseases readily transferred from one person to another are now spoken of as communicable. This term is supplanting the older term contagious.

Complement. A substance present in blood (ordinary or normal blood as well as immune blood) which completes (complements)

the dissolution or lysis of bacteria or other foreign cells invading the body.

Contagious. (See Communicable and Infectious.)

Cubic Centimetre. (c.c.) A small unit of size used in the metric system, now almost uniformly adopted for scientific measurements and descriptions. A cubic centimetre of water weighs one gram; probably a cubic centimetre can be visualized better by remembering that a cubic centimetre of such liquids as water contains about sixteen drops. For measuring liquids the word mil, (French, *mille*, a thousand) has been suggested as a substitute for cubic centimetre, as its form indicates its relation to the larger liquid unit of the metric system, the litre, a cubic centimetre or mil being one thousandth of a litre.

Diagnosis. A conclusion based upon a careful study of the characteristic signs or symptoms presented in a given case. Formerly a diagnosis was necessarily based upon clinical symptoms only; at present an examination of bacteriological or pathological material (*e.g.*, blood and feces in typhoid) is often made to confirm the clinical diagnosis.

Dialysis. The passage of soluble substances through membranes, either cell membranes, or artificial membranes such as dialyzing bags. (See Osmosis.)

Disinfectant. That which can de-

stroy infectious matter (bacteria, pus, etc.). Germicidal and sterilizing are stronger terms than disinfectant. Complete sterilization of dishes, utensils, bedding, etc., by heat or chemical germicides is rarely necessary as the organisms causing the common human diseases are usually killed long before the common and more resistant molds, and such harmless spore-producing bacteria as the hay bacillus. (See Germicidal.)

Endemic. (Literally, in or among the people.) Applied to any disease produced and continuously propagated by local conditions; *e.g.*, malaria and hookworm are endemic in certain areas of the United States.

Endotoxins. Toxins of such nature that they are not freely excreted from the living cell. (See Toxins.)

Enzymes. Substances produced by living cells which bring about definite chemical changes in those cells, their stored substances or their food materials. The growth and metabolism changes of all cells are dependent upon the action of such enzymes.

Epidemic. (Literally, upon the people.) Applied to sudden and widespread manifestations of disease, such as the recent "influenza epidemic." Epidemics differ from endemics in being sometimes attributable to the introduction of more virulent or entirely new organisms,

though physical conditions, famine, panic, etc., may be important factors in causing an "endemic disease to assume epidemic proportions."

Eustachian Tube. A tube connecting the cavity between the nose and the throat with the ear. Infection from the mouth or nasal region very often spreads into this tube and so into the ear, penetrating the spongy (mastoid) bone near the ear (mastoid abscess).

Exotoxins. Toxins which are easily or readily excreted from the cell producing them. (See Toxins.)

Exudate. The more or less liquid material found in a lesion or exuding from an inflamed area, such as the drip from nostrils, or the mucus or phlegm so characteristic of colds.

Feces. The discharges from the intestines or bowels.

Fibrin. A protein (globulin) substance present in the blood which helps form the clot in coagulating blood. Defibrinated blood is termed serum. (See Plasma and Serum.)

Filterable. Capable of being passed through a standard filter, such as the "porcelain" filters. (See illustration of filters in text.)

Food Sensitives. Individuals who experience such definite manifestations as hive-like eruptions, breathing difficulties or inflammation of the eye or nose mem-

branes, following the eating of one or more specific food substances, such as eggs, shellfish, and strawberries.

Flora. The plants present in a given region. A list of the kinds of bacteria found in a region, such as the mouth or intestine, would be termed the bacterial flora of that region.

Focus. (pl. Foci.) Site or seat of infection; the area of growth and multiplication of the invading organism. (See Lesion.)

Fomites. Substances capable of absorbing, holding and transporting infectious micro-organisms; particles of mucus, clothing, etc., come under this heading.

Furunculosis. A technical term for boils and similar infections.

Germicidal. A term used in reference to substances or agents (acids, heat, etc.) that can kill germs or micro-organisms. By implication (killing all germs) a germicide is a more powerful agent than a disinfectant, for a germicide must kill even resistant spores—such as mold spores—as well as the ordinarily less resistant organisms causing infection. (See Disinfectant.)

Germicidal substances, such as lysins, are produced by the body and may be demonstrated in the blood, *e.g.*, lysins against cholera organisms.

Globulin. See Protein.

Gonorrhea. A dangerous bacterial disease, affecting such delicate membranes as those of the eyes and the genital area. It is sometimes transferred through infected towels, bedding, and per-

sonal articles, as well as by personal contact.

Gram. A unit of weight. One cubic centimetre of pure water at its greatest density (4°C., or 39°F.) weighs one gram. The weight and bulk units of the metric system—gram and cubic centimetre—are, therefore, practically interchangeable for water measurements, but not for liquids lighter or heavier than water, or for most dry substances.

Hay-fever. A form of protein-sensitiveness, in which the patient is very susceptible to the presence of such substances as the pollen of certain plants, grasses, ragweeds, etc.

Hæmoglobin. The compound responsible for the coloring matter of red corpuscles and important as a carrier of oxygen. Ruptured or dissolved red corpuscles allow the escape of the colored hæmoglobin; tests involving such dissolution of red corpuscles are now in common use, *e.g.*, Wassermann test. (See Lysins.)

Hæmolysins. Lysins formed against and dissolving red blood cells, allowing the escape of the hæmoglobin. An animal injected with foreign red blood cells forms lysins against such foreign cells. Some bacteria (*Streptococcus*, *Hemophilus*) form similar lysins and, therefore, have very destructive action on red blood cells.

Hay bacillus. A common spore-producing organism, *Bacillus subtilis*, quite universally found on hay, in soil, water, etc.

Hemorrhagic. Showing evidence of hemorrhage; reddened or in

flamed by the accumulation, passage, or escape of blood.

Hookworm. A small round worm (*Necator*) which causes the hookworm disease. The hookworm is a many-celled animal as contrasted with the one-celled bacteria and protozoa with which most of this text deals.

Host. An organism that harbors another as a parasite. In typhoid or hookworm, man acts as host for the typhoid or hookworm organisms. In such diseases as malaria, man and the mosquito are alternately the host for the parasite.

Immune. Free from and resistant to disease. (See Immunity.)

Immune Blood. Blood from a person or other animal immune to a given disease; that is, blood from an animal which has recovered from a given disease—whether contracted “naturally” or as the result of the purposeful inoculation of the causal organism.

Immune Body. Part of a lysin—the “specific” part. Ambceptor is often used as a synonym for Immune Body. (See Lysin.)

Immunity. This is defined as freedom or exemption from; the literal meaning “not in service to,” carries the same implication, and gives an interesting parallel of the body as the slave of the disease organism. Immunity to a given type of infection may be due to antibodies produced in an earlier attack of that same disease. This is termed active immunity in contrast to passive

immunity where an individual lacking such active immunity may secure similar protection by injections of blood from another individual who is immune.

Incubation. When bacteria are kept at temperatures favorable for growth or multiplication, they are said to be incubated. We also speak of the initial time period when bacteria are multiplying in the body as the incubation period; this period extends from the time when the disease organism or organisms enter the body to the time when the symptoms appear and the patient “comes down” with the disease. The growth or multiplication of the organisms concerned, however, continues beyond this time—until the protective reactions of the body make such multiplication impossible.

Inert. Neutral; devoid of active chemical properties.

Infection. Disease organisms that multiply in the body cause infection. Infection may have a definite focus or be quite localized as in a boil or tooth abscess; or the infection may be more generally distributed, as in blood poisoning.

Infectious. Caused by disease-producing organisms; capable of producing disease through the transfer of such organisms, *e.g.*, infectious discharges from a wound or abscess. The old attempt to differentiate between infectious and contagious has become unnecessary since the adoption of the term communicable.

Inhibited. Prevented from grow-

ing or from multiplying in number. Chemicals, unfavorable temperatures, etc., may be used to inhibit bacterial growth or action.

Inflammation. A tissue reaction, of which the main symptoms are swelling and reddening due to the increased amount of blood in a given area; this may include the escape of one or more of the blood constituents, such as serum, into the inflamed tissue.

Inoculate. To introduce directly into the body, as into the skin, the abdominal cavity, or a blood vessel; used mainly in reference to the introduction of micro-organisms; curative substances (serum etc.) are more commonly spoken of as injected.

Intradermal. Into the skin, not underneath, as in subcutaneous.

Lesions. Injuries, diseased areas or morbid changes in organs or tissues. As examples of lesions we may cite an ulcer, a tubercular nodule, or a hemorrhagic area.

Lethal. Deadly; causing death, in a given or prescribed time.

Leucocytosis. An increase in the number of white corpuscles.

Leucopenia. A deficiency of white corpuscles.

Litre. A metric system unit for liquids, slightly larger than the quart; it consists of 1000 c.c. (See Cubic Centimetre).

Lymph-nodes. Small lymph-glands or collections of lymph tissue. Certain types of white corpuscles are produced in these lymph-glands; the inflammation

of certain lymph-nodes and the attendant pain (pressure) are due to the increased activity and blood content of such glands following infection.

Lymphocytes. Single-nucleated white corpuscles. Though not so generally active in bacterial destruction as the white corpuscles which have two or more nuclei, definitely helpful action is attributed to the lymphocytes in certain diseases, *e.g.*, tuberculosis.

Lysins. Dissolving substances or antibodies formed in the body as a reaction to bacteria (or other foreign cells, such as injected red blood cells). The action of a lysin is best explained by considering it as a double agent or substance: (1) The immune body and (2) the complement. Both factors are necessary for the lytic or dissolving action on foreign cells. Normal blood contains but one of these two factors, the complement; but a person recovering from such a disease as cholera, has also the second type, the "immune body," and therefore his blood can completely dissolve the cholera organism. (See *Hæmolysins*.)

Lysis. A dissolving or dissolution. (See *Lysins*.)

Macrophage. (See *White Corpuscles*.)

Medium. (pl. *mediums* or *media*.) Substances used to provide nutritive material for the growth and multiplication of micro-organisms; milk, beef broth and gelatine are examples of common mediums. For certain organisms

special kinds are used; these may contain egg for tuberculosis organisms, serum for diphtheria, blood for streptococci, etc.

Meninges. The enveloping membranes of the brain and spinal cord.

Meningitis. A disease of the meninges of the brain or spinal cord; the causal organism is usually the meningococcus organism but may be one of half a dozen other organisms—either characteristically disease-producing organisms, such as the pneumococcus or streptococcus organisms, or less virulent ones such as the colon bacterium.

Metabolic. (See Metabolism.)

Metabolism. A comprehensive term for all the cell changes, including the change of stable non-living food substances to complex unstable living material and the breaking down of that living material to simpler and more stable substances; metabolism includes all the upbuilding and energy-producing changes in a cell.

Metric System. A system of weights and measures very commonly used in Europe and now generally adopted in this country for scientific work. It is much simpler than our own systems the units being similar multiples or divisions of each other, varying by tens, hundreds, etc. The smaller units of the metric system are more suitable for the finer measurements than ours (See Gram, Cubic Centimetre.)

Micro-organisms. Organisms of

very small size demanding a microscope for detailed study. Bacteria and protozoa come under this general classification; members of many other groups (yeasts, molds) are small enough to be included.

Microphage. (See White Corpuscles.)

Mil. (See Cubic Centimetre.)

Minimal Lethal Dose. The smallest dose that uniformly causes death within a given or designated period.

Motility. The power of locomotion or moving from place to place. Flagella or organs which aid in locomotion are shown in the illustration of *Proteus vulgaris*.

Mucus. The viscid secretion of certain membranes; we are most familiar with it as the excessive secretion of such membranes as the nose and throat attending "colds."

Necrosis. The death or gangrene of a part of the body.

Necrotic. Dying; decomposing or in a gangrenous condition.

Non-pathogenic. Not capable of causing or producing disease. The hay bacillus is a non-pathogenic organism. (See Pathogenic.)

Normal Blood. The blood of a normal person, or one who has not had the disease under consideration. (See Immune Blood.)

Opsonic Index. The relative abundance of opsonins in an individual's blood as indicated by comparing the value of his serum

with that of a normal person.
(See Index.)

Opsonins. Antibodies produced as a reaction to invading organisms which aid the white corpuscles in digesting and destroying such invaders.

Optimum. Used to designate the condition or combination of conditions producing the best or most characteristic results; we speak, therefore, of optimum temperatures, optimum growth conditions, etc.

Organic. All organic substances contain carbon as an element; the term organic is, however, usually applied to substances which are or have been part of living organisms; flour, milk, serum and spores are organic substances in contrast to iron rust, table salt, and air, which are inorganic. Animals are dependent upon organic forms of food materials; while green plants often utilize certain organic substances formed by plant decay, they can exist on inorganic substances alone: Soil minerals, CO₂ and water. Most non-green plants (molds, bacteria) are, like animals, dependent upon organic types of food materials.

Osmosis. The term osmosis is now often limited to the passage of water through membranes, dialysis being used for the passage of the substances in solution, such as sugars, salts and acids. There is a constant exchange of substances in and out of all living cells by these two processes.

Parasite. (Literally, to live at another's table.) An organism

which lives upon another living organism. Tapeworms, the diphtheria organism, and such molds as those causing thrush and ringworm are examples of "human parasites."

Paratyphoid. An intestinal disease, or the organisms producing it; the symptoms and the causal organism resemble typhoid, therefore the prefix, *para*.

Passive Immunity. (See Immunity.)

Pathogenic. Capable of causing or producing disease; the tetanus or lockjaw organism is a pathogenic organism.

Pathogens. A term used to designate the micro-organisms which are capable of causing or producing disease.

Peritoneum. The delicate membrane which lines the abdominal cavity and covers the surfaces of the various organs in that region.

Peritonitis. An inflammation or infection of the peritoneum.

Phagocytes. Cells having the power of engulfing and destroying foreign matter, including bacteria or other foreign cells. Generally, when this term is used, the several-nucleated white corpuscles are meant. (See White Corpuscles.)

Phagocytosis. (See Phagocytes.)

Plasma. The fluid part of the blood, or blood minus the red and white corpuscles. (See Serum.)

Plasmolysis. A shrinkage or collapse of the cell attendant upon its loss of water.

Poliomyelitis. Infantile paralysis. A disease with serious lesions in

the spinal cord; the typical paralysis is due to the disintegration of spinal nerve cells ordinarily controlling the muscles in the areas (arm, leg) so paralyzed.

Polyvalent. (Literally, equal to many, or powerful in many ways.) A polyvalent serum is one which has the power of protecting against several varieties of organisms, having been produced by an animal vaccinated with all those varieties (that is, with a polyvalent vaccine).

Precipitate. (Literally, to throw or cast down headlong.) To cause to fall or settle more rapidly than would otherwise occur naturally by gravity.

Precipitins. Precipitating substances or antibodies formed as a reaction to the presence of foreign proteins in the blood stream; bacteria, the blood cells of another kind of animal, or even various ordinary food proteins, such as egg, milk, etc., may excite the production of appropriate precipitins. (See Fig)

Protein. The most important or foundation substance of all cells, or organisms, as indicated by the literal meaning of the word, *first in*. White of egg probably gives us a good common example of protein in the semi-liquid condition in which it exists as the ground work of most cells. Proteins differ in the number and proportion of the various constituents (iron, sulphur, phosphorus, magnesium, carbon, nitrogen,* etc.), the ways they are decomposed, their value as

cell building material, and their solubility in water, etc. Albumins and globulins are classed as simple proteins; peptones as derived proteins (decomposition products of simple proteins).

Protein Sensitiveness. Super- or hyper-sensitiveness to protein, including such varied phenomena as the sensitive or sensitized condition which evokes protective antibodies, and the disturbances known as hay fever and anaphylaxis.

Protozoa. The lowest group of animals, simple one-celled forms (such as amebas and trypanosomes). Protozoa represent the lowest animal divisions, just as bacteria form one of the lowest divisions of plants.

Ptomaines. These are alkaloid-like substances (amino-acids) formed in the decomposition of organic matter, especially proteins. Some of them are poisonous, though most ptomaines are not. The more complex ptomaines, especially those containing oxygen, are the more poisonous ones. The simpler ones such as methylamine, (CH_5N), are not toxic, while muscarin ($\text{C}_5\text{H}_{15}\text{NO}_3$) and sepsin ($\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$) are very toxic.

Pulmonary. Relating to or affecting the lungs, as pulmonary tuberculosis.

Pyemia. The distribution of infectious material from an initial focus by the blood may result in the establishment of several other infection areas, a general pyemia.

Pyogenic. Pus-forming or capable of causing infection.

Receptors. Chemical or atomic groups which are conceived as affording cell combinations not only with food but with injurious materials, such as toxins and split-proteins. Advanced texts on immunity classify antibodies as falling into three receptor groups, varying in their susceptibility to heat, etc.

Saprophyte. An organism which lives on organic material, but not on a living host. Bacteria or molds living on bread, dead fish, milk, are saprophytes. Many parasitic organisms may become adapted to a saprophytic mode of existence, and, *vice versa*, most disease organisms can be grown as saprophytes on ordinary laboratory media. (See Parasites.)

Sepsis. (See Septic.)

Septic. Undergoing or showing signs of sepsis or decay. A septic wound has decay-producing organisms growing in it; pus and "proud flesh" are indications of such septic conditions.

Sensitized vaccine. Vaccine modified by the addition of its specific antibodies or anti-serum containing one or more types of antibodies. "Toxin-antitoxin" used to prevent diphtheria is really such a sensitized vaccine. (See Index.)

Septicemia. When the organisms causing infection actually multiply in the blood stream, the condition is called a septicemia. (See Bacteria and Pyemia.)

Sero-vaccine. Vaccine modified by the addition of anti-serum, a sensitized vaccine.

Serum. Serum differs from plasma, the liquid part of the blood, in lacking not only the red and the white corpuscles but the fibrin of the plasma as well. When blood clots the resulting yellowish liquid is the serum, the solid clot containing the enmeshed corpuscles and fibrin. (See Whole Blood.)

Serum Sickness. A term covering such manifestations as skin eruptions, fever, rheumatic pains, and inflammation of the joints and glands occasionally caused by the injection of serum, such as horse serum.

Species. A group of similar individuals, all resembling each other, and all differing in the same respects from other members or species of the larger group or genus to which they all belong. The genus *Streptococcus* contains such different species, for example, as *Str. viridans* (forming green pigment on blood agar), and *Str. pyogenes*, (dissolving the red blood cells and forming a transparent area on blood agar). All the members of the species *viridans* resemble the rest of the genus in their chain-forming method of growth, but they differ from them in producing the green pigment. These green-producing organisms may differ from each other in minor or less constant ways (size, chain length) than they differ from the other species;

strains showing such lesser differences are spoken of as *varieties*. (See Type.)

Spirillum. (pl. *Spirilla*.) One of the classes of spiral-shaped bacteria is called *Spirillum*; *Vibrio* includes the shorter spirals, such as the cholera organism.

Spirochæta. The protozoa include a group of spiral shaped organisms called *Spirochæta*. The spiral protozoa are larger and longer—with more spirals or turns than the spiral bacteria. Syphilis is caused by one of these spiral protozoa.

Spirochetes. Anglicized form of *Spirochæta*.

Split-proteins. A term used in connection with the decomposition of proteins, especially bacterial proteins, into irritating substances, termed "poisonous split-proteins." (See Index.)

Spore. Some species of bacteria form spores—inactive stages—in their life history or development. Spores are quite resistant to most unfavorable conditions, and are usually formed in response to such environmental influences or changes.

Sputum. Mucus accumulating in or ejected from the mouth or throat.

Stains. Special dyes or stains are used to color bacteria so they may be seen more definitely. No real progress was made in identifying bacteria until after this application of Perkin's discovery of anilin dyes.

Sterile. Free from micro-organisms. Substances may be ren-

dered sterile by the application of heat, chemicals, electricity, etc. Instruments, bandages, etc., are made sterile by such methods. It is not always possible to make wounds and body tissues sterile. In such cases the growth of undesirable or pathogenic organisms must be prevented by the use of inhibiting or antiseptic agents.

Sterilize. To free from all micro-organisms by such agents as heat or chemicals. A stronger term than disinfect, which has as its aim only the killing of the infections or disease-producing organisms which may be present.

Streptococci. Coccus or globular bacteria which adhere in chains, related to many types of human disease or infection, such as abscesses, erysipelas and blood-poisoning.

Subcutaneous. Underneath the skin.

Supernatant. Rising or floating over or above, as the liquid over a precipitate or sediment.

Symbiosis. A living together of dissimilar organisms, each type of which contributes thereby some necessary factor or condition for the welfare of the other.

Syphilis. The most dangerous of the venereal diseases, sometimes transferred by common drinking glasses, towels, bedding, or personal articles, as well as by personal contact.

Tetanus. The lockjaw organism, *Clostridium tetani*, or the disease caused by it.

Toxins. (Literally, an arrow, and

referring to the poison used on arrow tips.) Poisons formed by bacteria in their ordinary living or metabolic processes. In certain disease organisms, such as diphtheria, these toxins may be excreted freely by the bacteria (exotoxins) and accumulate in the blood; in most organisms toxins, if formed, are not liberated freely from the living cell but are freed only on the death or disintegration of the cells.

Toxoid. An altered toxin, having less toxic or poisonous effects.

Toxon. A secondary or less well-known toxin, differing from the primary or better-known toxin formed by a given organism.

Tropins. A term used to distinguish the opsonins in immune blood from normal opsonins—or the opsonins present in normal blood.

Trypanosomes. A group of protozoan or one-celled animals which cause various diseases in animals but none common in man in this country.

Tuberculin. A preparation of killed tuberculosis organisms used chiefly as an exciting agent to demonstrate whether or not a given individual is sensitive to that organism.

Tuberculosis. A disease of the lungs, glands, bones, etc., which in man is caused by one of two organisms: the ordinary human type of tuberculosis, or, a related species, the bovine type.

Type. A characteristic example; a specimen that embodies the

characteristics of the species or group. Recently, in bacteriology, the word type has been used more in the sense of the varieties or lesser groups of a given species, especially those based upon serum tests (agglutination, etc.); this is the meaning underlying such expressions as "pneumococcus, type 1".

Vaccine. (From *vacca*, a cow, because the first vaccine—against smallpox—was obtained from cows.) Vaccine is a term used for substances, such as bacteria, which are inoculated into the body to excite protective substances against the related disease. Some vaccines are made of living, others of dead organisms; if living organisms are used, they are first weakened in some way to lessen their effect on the body. Even dead organisms may be modified to lessen the serious effects upon the tissues, to favor absorption, etc., as in sensitized vaccines, and lipo-vaccines.

Vibrio. See *Spirillum*.

Virulence. Power to produce injury in a host by growth and the production of poisonous substances; virulence varies with the invasive power of the organism as well as the strength and kind of injurious substances produced. (See *Virulent*.)

Virulent. (Literally, full of poison.) Highly pathogenic or extremely toxic or poisonous. (See *Virulence*.)

Virus. A term often used to in-

clude or designate the causal agents of disease, especially if the organism has not been seen or identified. The material used to vaccinate against smallpox is commonly spoken of as smallpox virus. Similarly we speak of the virus of hog-cholera, and of foot-and-mouth disease. When the causal organism is known, it is usually spoken of by its group or generic name (*Streptococcus*, *Trypanosoma*, *Spirillum*, etc.) or by such general names as bacterium or protozoan.

Vital Resistance. The resistance of animals or other individuals against such untoward agents as infection and fatigue. Upon the individual's resistance depends his freedom from disease or the severity of the attack, and the rate or chance of recovery. This resistance is in great part dependent upon the activity of the white corpuscles and the production of such antibodies as anti-toxins and opsonins.

White Corpuscles. White corpuscles, though relatively few in number when compared with the red, are very important; they help in blood clotting, in keeping a protein balance in the blood, etc. The white corpuscles vary more than the red in size and activity; although several kinds of white corpuscles are found in all human blood, they may, for our purposes, be divided into two main groups, those that contain but one nucleus and those that contain several nuclei. Those

possessing two or more nuclei have the power of surrounding or enveloping and finally digesting bacteria, thus protecting the body against invading organisms. Sometimes dozens of bacteria may be seen inside one white corpuscle. Consult the index for illustrations of an example of this destructive phagocytic action. These smaller white corpuscles are sometimes termed microphages or microphagocytes to distinguish them further from the other larger cells having phagocytic action such as the single-nucleated lymphocytes. White corpuscles active in bacterial destruction are sometimes spoken of as bacteriophages.

This elementary presentation does not warrant the discussion of the destructive (lytic) "bacteriophage principle" demonstrated by current investigations in successive cultures or generations of staphylococci, typhoid, etc.; this bacterial destruction is variously explained as a "bacterial disease," an enzyme reaction of the host tissues, and (despite the inhibiting and dissolving action on the respective bacteria) a product of the bacteria themselves.

Whole Blood. Literally whole blood, without the corpuscles subtracted. Blood from which the corpuscles have been removed is spoken of as plasma; if the fibrin is also taken out the resultant liquid is called serum. (See Plasma and also Serum.)

LIST OF INFECTIONS, AND CAUSAL ORGANISMS DISCUSSED IN THIS TEXT*

OF PLANT ORIGIN

<i>Acne</i>	<i>Fusiformis acne</i> (<i>Bacillus acne</i>)
<i>Anthrax</i>	<i>Bacillus anthracis</i>
<i>Bacillus acidophilus</i>	<i>Lactobacillus acidophilus</i> (<i>Bacillus acidophilus</i>)
" <i>acne</i> (See <i>Acne</i>)	
" <i>anthracis</i> (See <i>Anthrax</i>)	
" <i>bulgaricus</i>	<i>Lactobacillus bulgaricus</i> (<i>Bacillus bulgaricus</i>)
" <i>coli</i>	<i>Bacterium coli</i>
" <i>edematis</i> (<i>maligni</i>) (See <i>Malignant edema</i>)	
" <i>maeli</i> (See <i>Glanders</i>)	
" <i>pertussis</i> (See <i>Whooping-</i> <i>cough</i>)	
" <i>pestis</i> (See <i>Plague</i>)	
" <i>subtilis</i>	<i>Bacillus subtilis</i>
" <i>typhosus</i> (See <i>Typhoid</i> <i>fever</i>)	
<i>Bacterium coli</i>	<i>Bacterium coli</i>
" <i>dysenteriae</i> (See <i>Dysentery</i>)	
" <i>paratyphosum</i> (See <i>Para-</i> <i>typhoid fever</i>)	
" <i>pneumosintes</i> (See <i>Influ-</i> <i>enza</i>)	
" <i>pneumoniæ</i> (See <i>Pneu-</i> <i>monia</i>)	
<i>Botulism</i>	<i>Clostridium botulinum</i> (<i>Bacillus botulinus</i>)
<i>Cholera</i>	<i>Vibrio cholerae-asiatica</i> (<i>Vibrio cholerae</i>)

*The bacteria are listed (second column) by the names in the Generic Index in the 1920 report of the Society of American Bacteriologists.

Clostridium botulinum (See Botulism)	
" edematis (See Malignant edema)	
" tetani (See Tetanus)	
" Welchii (See Gas gangrene)	
Diphtheria	<i>Corynebacterium diphtheriæ</i> (<i>Bacillus diphtheriæ</i>)
Diplococcus intracellularis meningitidis (See Meningitis)	
" meningitidis (See Meningitis)	
" pneumoniæ (See Pneumonia)	
Dysentery	<i>Bacterium dysenteriæ</i> (<i>Bacillus dysenteriæ</i>)
Fowl cholera	<i>Pasteurella cholerae-gallinarum</i>
Gas gangrene	<i>Clostridium Welchii</i> (<i>Bacillus Welchii</i>) (Other organisms may be found in gangrenous conditions)
Glanders	<i>Pfeifferella mallei</i> (<i>Bacillus mallei</i>)
Gonococcus (See Gonorrhea)	
Gonorrhea	<i>Neisseria gonorrhæa</i> (<i>Micrococcus gonorrhæa</i> ; <i>Gonococcus gonorrhæa</i>)
Hay bacillus	<i>Bacillus subtilis</i>
Hemophilus influenzae (See Influenza)	
" pertussis (See Whooping-cough)	
Infantile paralysis (See Poliomyelitis)	
Influenza	<i>Hemophilus influenzae</i> (<i>Bacillus influenzae</i>) Recently a filterable organism <i>Bacterium pneumosintes</i> , has been found.
Lactobacillus	<i>Lactobacillus acidophilus</i> " <i>bulgaricus</i>

Leprosy	<i>Mycobacterium lepræ</i> (<i>Bacillus lepræ</i>)
Lockjaw (See Tetanus)	
Malignant edema	<i>Clostridium edematis</i> (<i>maligni</i>) (<i>Bacillus edematis</i> [<i>maligni</i>])
Meningitis	<i>Neisseria meningitis</i> or <i>intracellularis-meningitidis</i> (Earlier classified under <i>Micrococcus</i> , <i>Diplococcus</i> , and <i>Meningococcus</i> .) Many other organisms may cause meningitis such as the tuberculosis, influenza and typhoid organisms.
Meningococcus (See Meningitis)	
Micrococcus gonorrhea (See Gonorrhea)	
" meningitidis (See Meningitis)	
Mumps (Causal organism uncertain)	
<i>Mycobacterium lepræ</i> (See Leprosy)	
" tuberculosis (See Tuberculosis)	
<i>Neisseria gonorrhæ</i> (See Gonorrhea)	
" meningitidis (See Meningitis)	
Paratyphoid fever	<i>Bacterium paratyphosum</i> var. A or B, (<i>Bacillus paratyphosus</i> var. A or B).
" A	
" B	
<i>Pasteurella pestis</i> (See Plague)	
Pertussis (See Whooping-cough)	
<i>Pfeifferella mallei</i> (See Glanders)	
Plague	<i>Pasteurella pestis</i> (<i>Bacillus pestis</i>)
Pneumonia	<i>Bacterium pneumoniae</i> (Formerly usually classed under <i>Diplococcus</i> , <i>Micrococcus</i> or <i>Pneumococcus</i> .) Many other organisms may cause pneumonia, notably <i>Streptococcus pyogenes</i> and <i>Hemophilus influenza</i> .

Poliomyelitis	"Globoid bodies" of Flexner (Classification as plants uncertain)
Proteus vulgaris	<i>Proteus vulgaris</i>
Staphylococcus	<i>Staphylococcus aureus</i> <i>Staphylococcus pyogenes-aureus</i>
Streptococcus	<i>Streptococcus mucosus</i> <i>Streptococcus pyogenes</i> <i>Streptococcus viridans</i>
Tetanus	<i>Clostridium tetani</i> (<i>Bacillus tetani</i>)
Tuberculosis	<i>Mycobacterium tuberculosis</i> (<i>Bacillus tuberculosis</i>)
Typhoid fever	<i>Bacterium typhosum</i> (<i>Bacillus typhosus</i>)
Whooping-cough	<i>Hemophilus pertussis</i> (<i>Bacillus pertussis</i>)

OF ANIMAL ORIGIN (Mainly Protozoa)

Hookworm	<i>Anchylostoma americanus</i> (<i>Necator americanus</i>)
Malaria	<i>Plasmodium malarie</i>
Measles (Causal organism unknown)	
Nagana	<i>Trypanosoma Brucei</i>
Negri bodies (See Rabies)	
Rabies	"Negri bodies"
Smallpox (Causal organism unknown)	
Syphilis	<i>Treponema pallidum</i> (<i>Spirochæta pallida</i>)
Spirochæta pallida (See Syphilis)	
Treponema pallidum (See Syphilis)	
Trypanosoma (See Nagana)	
Yellow fever	<i>Leptospira icteroides</i>

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INDEX

In this index micro-organisms are listed under the names usually used in conversation: typhoid, diphtheria, ameba, streptococcus, etc.; for their scientific names see the table of synonyms, p. 230. A list of the organisms illustrated in this text is given under the index heading Micro-organisms. Black type is used throughout to distinguish references to illustrations from subject matter.

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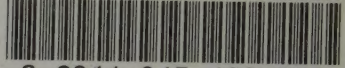
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